Thesis in pharmacology for the degree *Candidata pharmaciae*

CICLOSPORIN A – DEVELOPMENT OF A PHARMACOKINETIC POPULATION MODEL

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I ACKNOWLEDGEMENT

This thesis in pharmacology is a part of the degree *cand.pharm.* The present work was conducted at the Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, from November 2006 to November 2007.

First of all, I would like to thank Professor Anders Åsberg for his generous guidance and contribution. Your interest and enthusiasm been greatly appreciated during this year. I would also thank you for the opportunity to participate at the NONMEM course in Gent, Belgium.

I would also thank Ph.D. student Pål Falck for all the help during this year, and for being available for all kinds of questions. Your assistance has been invaluable! Also, thanks for a great trip to Gent, Belgium.

Thanks to everyone at Department of Pharmaceutical Biosciences for a friendly environment and many delicious cakes during the year. And finally, I would like to thank the co-students for a social atmosphere all through the year.

Oslo, 13.November 2007

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III ABBREVIATIONS

ABCB1	Gene sequence that codes P-gp
AUC	Area under the time-concentration curve
AUC_{0-12}	Area under the time-concentration curve between C_0 and 12 hours post dose.
AUC_{0-4}	Area under the time-concentration curve between C_0 and 4 hours post dose.
C_0	Concentration prior to dose (through levels)
C_2	Concentration 2 hours post dose
CI	Confidence interval
CL	Apparent clearance
C_{max}	Maximum concentration of drug
CP	Cyclophilin
CRCL	Creatinine clearance
CsA	Ciclosporin A
CV	Coefficient of Variation
CYP	Cytochrom P-450
F	Bioavailability
GOF	Goodness of fit
i.v.	Intravenous
IL-2	Interleukin-2
IPRED	Individual predicted concentrations
k_a	Absorption rate constant
k_{tr}	Transfer rate constant between the sequential compartments in the Erlang model
MAP	Maximum *a posteriori* probability
MAPE	Mean absolute prediction error
MPE	Mean prediction error
NFAT	Nuclear factor of activated T-lymphocytes
OBS	Observed concentrations
OFV	Objective function value
p.o.	Per oral

P-gp	P-glycoprotein
PRED	Predicted concentrations
Q	Intercompartment clearance
r	Coefficient of correlation
r^2	Coefficient of determination
RBC	Reed blood cells
RES	Residual error (OBS-PRED)
SD	Standard deviation
STS	Standard two-stage
TDM	Therapeutic drug monitoring
T-lymphocytes	Thymus lymphocytes
V_c	Distribution volume in central compartment
V_d	Apparent volume of distribution
V_p	Distribution volume in peripheral compartment
WRES	Weighted residual error (RES expressed in fractions of population SD units)
WT	Weight

IV ABSTRACT

Background

Ciclosporin A (CsA) is an important part of the immunosuppressive regimen in the treatment of renal transplant patients. CsA is typified by a great inter- and intraindividual pharmacokinetic variability, and narrow therapeutic window. Concentrations over the therapeutic window are associated with serious side effects, while concentrations under the therapeutic window are associated with risk of organ rejection. Therapeutic drug monitoring of CsA is therefore necessary.

A pharmacokinetic population model predicts individual pharmacokinetic parameters not only based on patient observations, but also upon population data. The large pharmacokinetic variability of CsA seen in the population as well as significant patient demographics are implemented in such a model. A pharmacokinetic population model of CsA can therefore be a valuable tool used to optimize CsA dosing. The purpose of this study was to develop a pharmacokinetic population model for CsA.

Methods

Twelve hour concentration-time profiles of CsA from 17 renal transplant recipients were used to develop a pharmacokinetic population model using the nonlinear mixed effect approach as implemented in NONMEM. Different compartment models and especially different absorption processes were examined in order to find the best pharmacokinetic population model for CsA. Influence of covariates on the pharmacokinetic parameters was examined in accordance with traditional methods. The complete model was validated using both internal and external methods.

Results

A 2-compartment model with Erlang distribution as an absorption process was found to describe the pharmacokinetic data best. For the Erlang distribution, the optimal number of lag compartments placed upstream to the central compartment was six. Among the different covariates investigated, only age had a significant influence on the estimation of clearance.

The internal validation process found no individuals with large influence on the pharmacokinetic parameters and the model showed great robustness. In addition, the population model was able to predict individual AUC_{0-12} in patients excluded from the dataset using limited samplings points within the absorption phase.

An external validation in 10 new renal transplant recipients showed that the pharmacokinetic population model also could predict individual AUC_{0-12} in an external population with same accuracy as in the internal validation process.

Conclusion

A 2-compartment model with Erlang distribution as an absorption process and age as a covariate on clearance described the CsA data best. This population model provides a good basis for the development of a model that can serve as a Bayesian prior when designing dosing regimens in new kidney transplant patients.

1 INTRODUCTION

1.1 POPULATION PHARMACOKINETICS

1.1.1 Introduction

Population pharmacokinetics is an approach to quantify determinants of drug concentrations in a population of patients [1]. It can be defined as the study of variability in plasma drug concentrations among individuals representative for the target population group receiving the drug [2]. The use of population approaches for doing pharmacokinetic analyses has increased during the last 15 years [3].

In contrast to traditional pharmacokinetic analyses, the population approach encompasses some important features. Population pharmacokinetics seeks to obtain relevant pharmacokinetic information in patients who are representative of the target population. In addition it identifies and quantifies the sources of variability that contributes to differences between expectations and outcome. The variability is categorized as interindividual and residual [4, 5].

Interindividual variability is the biological variability that exists between subjects. Searching for covariates that can account for some of the interindividual variability is another important feature of population pharmacokinetics. Covariates can be patient demographic features such as age, gender and body weight, environmental factors, genetic phenotypes, drug-drug interactions and physiologic factors such as renal impairment [4, 5].

Residual variability is variability due to errors in concentration measurements, misspecifications of the model, inexplicable day-to-day or week-to-week variability (i.e. interoccasion variability) and intraindividual variability. Intraindividual variability is differences between the predictions of the model *for the individual* and the measured observations. Population pharmacokinetics also has the important feature of quantitatively estimate the residual variability in the patient population, which may give important information regarding drug efficacy and safety [4, 5].

Population pharmacokinetics is often used in both drug development and individual dosing regimens. In drug development, population pharmacokinetics can help designing dosing

guidelines [6]. The approach is recommended in the US Food and Drug Administration (FDA) guidance for Industry as part of the drug development process [7]. For individual dosing regimens, population pharmacokinetics is useful in Bayesian approaches for estimation of individual pharmacokinetic parameters used in therapeutic drug monitoring [8]. In general, population pharmacokinetics is especially useful when working with drugs that have narrow therapeutic window and show large pharmacokinetic variability.

Pharmacokinetic analyses can be model-dependent or -independent. Non-compartment approaches are model independent, which means that no assumption is made of any specific compartment model. Model independent analyses are often used to calculate basic pharmacokinetic parameters, which can be used as primary estimates in the population models. Model dependent analyses are often a more accurate physiological description of the data, where the models represent the body as a system of compartments.

1.1.2 The concept of compartments

In pharmacokinetic population modeling the body can be described in terms of compartments. A compartment is not a real physiologic or anatomic region. It represents a tissue or group of tissues that have similar blood flow and drug affinity. Within each compartment the drug is presumed to be uniformly distributed and to reach distribution equilibrium immediately [9].

The simplest pharmacokinetic model consists of one compartment, which assumes that changes in the plasma level of a drug reflect proportional fast changes in tissue drug level [9]. However, not every drug equilibrates rapidly throughout the body as assumed for a one-compartment model. In multicompartment models the drug distributes into the central compartment and one or more tissue/peripheral compartments. The central compartment represents the blood, extracellular fluid and highly perfused tissues that rapidly equilibrate with the drug. The tissue/peripheral compartment represents tissues where the drug equilibrates less rapidly [9].

The number of compartments required to describe the distribution of the drug equals the number of exponential terms needed to describe the plasma concentration-time curve [10]. Thus, a 2-compartment model is needed when the plasma concentrations are best fitted with a bi-exponential equation.

The pharmacokinetic parameters can all be part of the compartment model, as indicated in figure 1. The rate constants for the transfer between compartments are referred to as micro constants or transfer constants. Elimination is often assumed to occur from the central compartment, since the major sites of elimination are the kidney and the liver that are highly perfused with blood, and hence most often exerts fast distribution equilibrium. If the drug is eliminated at a constant rate, which means that the fractional rate of decline ($\Delta C/\Delta t$ versus C) increases with time, the elimination kinetic is called zero order. In contrast, if the fractional rate of decline is constant, the elimination is assumed to be first order [10].

When the drug is administrated extravascularly, absorption is characterized by an absorption rate constant, k_a, and a corresponding absorption half-life. The absorption, like elimination, can occur with zero or first order kinetics [10].

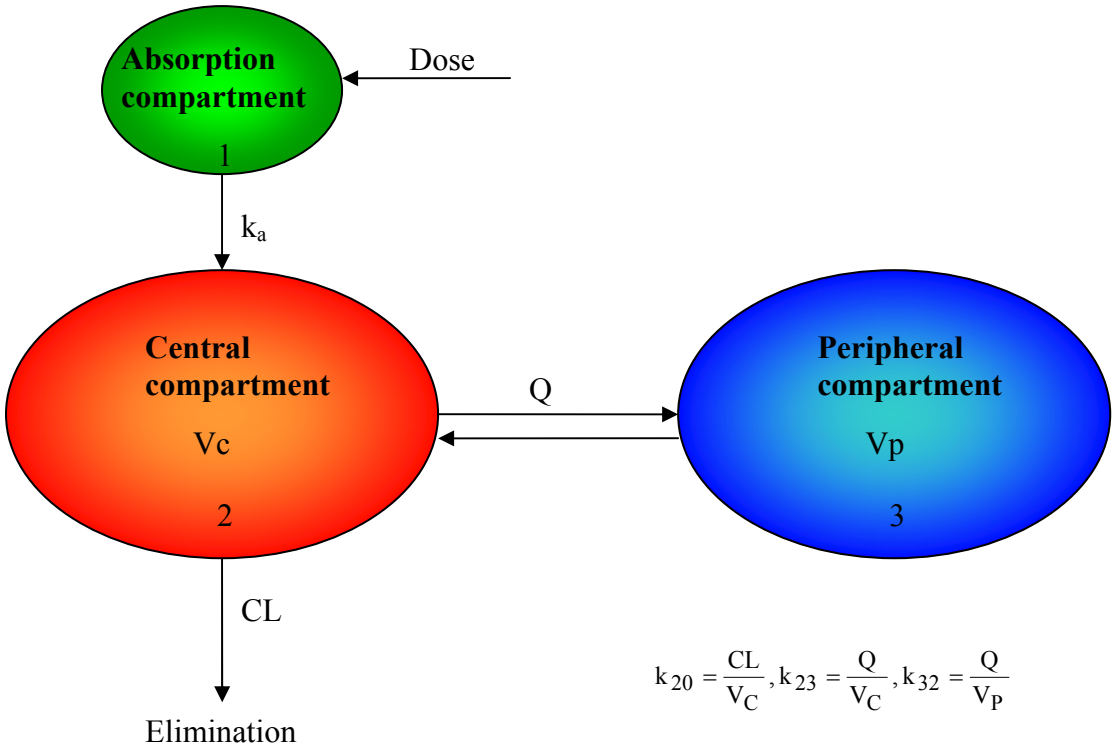

Figure 1: 2-compartment model with extravascular administration. The drug is absorbed inversely from compartment 1 into compartment 2, distributes between compartment 2 and 3 and is eliminated from compartment 2.

Ka: absorption rate constant, **Vc**: distributjion volume in central compartment, **CL**: apparent clearance, **Vp**: distribution volume peripheral compartment, **Q**: intercompartment clearance, $\mathbf{k_{20}}$: elimination constant from compartment 2, $\mathbf{k_{23}}$, $\mathbf{k_{32}}$: rate constant between the compartments indicated.

1.2 MODELING APPROACHES

Modeling approaches are either parametric or nonparametric. Parametric models have continuous parameter distribution, and the distribution is assumed to be either normal or lognormal. The parametric methods obtain means and standard deviations (SD) of the parameters, and correlations between them [11]. Nonparametric methods have no assumptions about the shape of the parameter distribution, which mean that no specific parameters such as means and SDs are used to describe the distribution of the parameters within a population *a priori*. The shape of the distribution is instead exclusively determined from the population raw data [12].

The two most common methods for doing population pharmacokinetic analysis is the standard 2-stage (STS) approach and the nonlinear mixed-effects approach, which are both parametric methods [11].

1.2.1 Standard 2-stage (STS) approach

The standard 2-stage (STS) approach is the traditional method based on data-rich situations. The first stage involves estimation of individual pharmacokinetic parameters (and the correlations between them), using a method such as weighted nonlinear least squares. In the second stage the individual measurements are used to calculate the population mean and SD [11, 12].

STS has the disadvantages of requiring at least one serum concentration data point for each parameter to be estimated, and does not consider variance of point estimates [11, 12]. STS gives poor predictions of parameters in situations with sparse data. However, this method is easy to implement and quick to run.

1.2.2 Nonlinear mixed-effects approach (1-stage approach)

The nonlinear mixed-effects approach considers the population sample, rather than the individual, as a unit for estimation of the distribution of parameters and the covariance and correlations between them [13]. It is called a 1-stage approach since all data of all individuals are analyzed at once. This method also works with only one measurement per patient [11, 12], and takes the variability within and between the individuals into account [13]. Other

advantages of the nonlinear mixed-effects method over STS are that it finds the best set of parameters and one can perform formal testing of covariates. However, it has the disadvantages of being more difficult to implement and slower to run compared to STS.

The first true nonlinear mixed-effects modeling program introduced was NONMEM. See section 1.3.

1.2.3 Bayesian procedure

Bayesian procedure is a common method to estimate a patients own particular set of parameters [8], where the focus is moved from the typical patient to view the patient as unique. The results of a population analysis provide information to estimate an individualized dosing regimen, based on expected mean values of the parameters and estimates of the variability [1]. This approach balances the uncertainty in the individual parameter against uncertainty in the observations; the *posterior* are highly influenced of the probability that the *prior* is true.

1.3 NONMEM

1.3.1 Introduction

A general approach to use patient data to account for some of the pharmacokinetic /pharmacodynamic variability among a patient group was introduced as early as 1972 by Sheiner et al. [14]. They suggested using non-linear mixed effects regression models to quantify inter- and intraindividual variability. The concept developed further into a computer program, NONMEM, which was first released in the early 1980s by Lewis Sheiner and Stuart Beal [15]. Besides being the oldest, NONMEM is probably the most widely used population analyse program today [16].

NONMEM uses several building blocks to develop a mathematical representation (model) of experimental data arising from an unknown underlying process. One building block is the structural model that describes the basics of the process being examined. Other building blocks describe the random effects [15]. See sections 1.3.2 and 1.3.3.

1.3.2 Fixed effects in NONMEM

The structural part of the model contains measurable population parameters and known patient characteristics. This is the explained part of the model [1]. Fixed effects are the population parameters, which in NONMEM are called theta (θ). The thetas define the average value for the population parameters, such as CL and V_d, and/or the average relationship between the population parameters and patient cofactors, such as weight and renal function [15].

1.3.3 Random effects in NONMEM

NONMEM estimates the distribution of the random effects, which is typically normal with a mean zero and a variance. The building block for interindividual variability in NONMEM is eta (η) with a variance called omega squared (ω^2). The building block for intraindividual variability in NONMEM is epsilon (ε) with a variance called sigma squared (σ^2) [15].

Interindividual variability: $\eta = N(0, \omega^2)$ (1)

Intraindividual variability: $\varepsilon = N(0, \sigma^2)$ (2)

Random effects are implemented in NONMEM by using variance models. The most common variance models are additive, proportional and log normal. These models are applied to both inter- and intraindividual variability [15].

Table 1: Variance models for random effects.

Additive variance model	Value = Predicted + Error
Proportional variance model	Value = Predicted * (1+Error)
Log normal model	Value = Predicted * Exp (Error)

1.3.4 Maximum Likelihood Estimation

NONMEM uses maximum likelihood estimation when calculating the objective function value (OFV). OFV is an indication of how likely the present observations would have been observed, given that the model is true. NONMEM maximizes likelihood by minimizing -2 log likelihood [15].

The probability/likelihood of one observation is given by:

$$L = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{1}{2\sigma^2}\left(Y-\hat{Y}\right)^2} \tag{3}$$

Where Y is the measured observation, $\hat{Y}$ is the prediction of that observation by the model and σ^2 is the variance of the model. When expanding this to n observations and using -2 log instead of just L, one get the -2 log likelihood equation:

$$-2\log(L) = n\log\left(2\pi\right) + \sum_{i=1}^{n}\left(\log\left(\sigma_i^{\,2}\right) + \frac{\left(Y_i - \hat{Y}_i\right)^2}{\sigma_i^{\,2}}\right) \tag{4}$$

The maximum likelihood principle states that one should choose those parameter estimates that correspond to the maximum of this likelihood function. This is because these particular parameter estimates render the observed data most probable to be “true” [17]. However, the best model is not necessarily the model with the lowest OFV, and different datasets can not compare OFV in absolute terms. A complex model with the lowest OFV always has to be justified with significant better fit of the data. Otherwise a more simple and faster model is preferred, especially if the model is to be used in clinical practise.

1.4 CICLOSPORIN A

1.4.1 Introduction

Ciclosporin A (CsA) is a lipophilic cyclic polypeptide containing 11 amino acids. It was isolated for the first time from the fungus *Toly pocladium inflatum* found in Hardangervidda. CsA's immunosuppressive properties were discovered in 1972 [18], and was introduced into the marked as an immunosuppressive agent in the beginning of the 1980s [19].

CsA has been an important immunosuppressive agent in clinical practice since its introduction [19]. CsA led to an improvement in transplant graft outcome [20, 21], and improved the ability to transplant hearts [22]. However, CsA treatment is also associated with serious side effects such as nephrotoxicity, hypertension, dyslipidemia and development of diabetes [23-26]. Due to the facts that CsA has a narrow therapeutic window and displays extensive inter- and intravariability in the pharmacokinetics (figure 2), routine therapeutic drug monitoring of CsA is necessary [19, 27], and the use of a population model would probably be of great value.

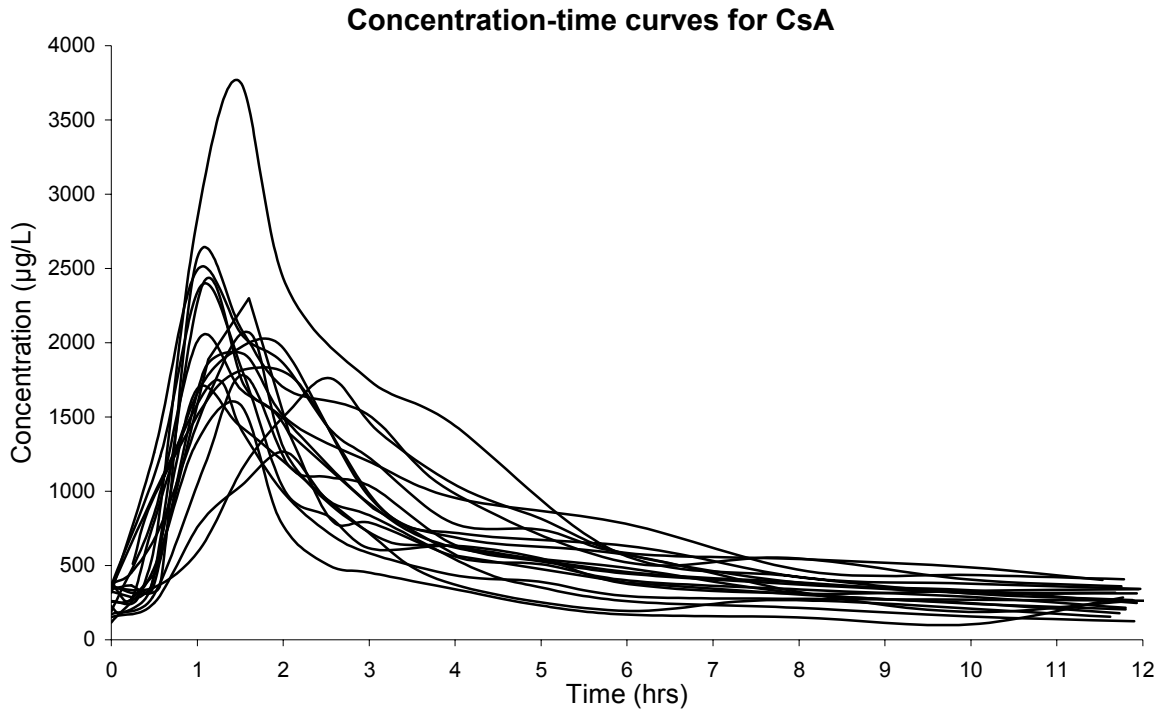

Figure 2: Interindividual variability.

Interindividual variability within the kidney transplant patients used to build the pharmacokinetic population model in this thesis (n=17).

1.4.2 Mode of action

The mechanism of the immunosuppressive action results primarily from a selective suppression of T-lymphocyte activation. CsA inhibits the phosphatase activity of calcineruin via formation with cyclophilin, an intracellular protein in T-lymphocytes. This action prevents translocation of the nuclear factor of activated T-lymphocytes (NFAT), which is necessary for transcription of lymphokine genes, most notably the major T-lymphocyte growth factor interleukin-2 (IL-2) [28]. Thus, administration of CsA leads to blockage of transcription of lymphokine genes, which are essential for the differentiation and proliferation of T-lymphocytes.

1.4.3 Absorption

The absorption of CsA after oral administration is unpredictable and shows large interpatient variability, and is characterized by a lag phase followed by rapid absorption. The site of absorption is predominantly the small intestine [29]. Due to its lipophilicity the absorption is dependent of bile flow, but is also affected by gut motility, food and time after transplantation [30]. With the conventional oral formulation of CsA (Sandimmun®), the bioavailability ranges from 1% to 89% [30]. A microemulsified formulation of CsA (Sandimmun Neoral®) improved the bioavailability and reduced the variability of gastrointestinal absorption [31]. This formulation has been used since mid 1990's. A higher correlation between CsA dose and AUC has been shown with Sandimmun Neoral® compared to the conventional formulation [32]. However, there is still a large variation in the absorption of CsA.

1.4.4 Distribution

CsA is highly distributed to extravascular tissues, and has high affinity to blood cells and plasma components. Due to the lipophilic nature of CsA, the drug accumulates predominately in fat-rich organs such as liver, adipose tissue and lymph nodes [33]. About 50% of CsA in blood is bound to erythrocytes, 15% to leukocytes and 33% to plasma proteins and lipoproteins. In the plasma fraction, lipoproteins are the major complexing constituents for CsA [34]. The distribution of CsA in erythrocytes is dependent on temperature [35] and concentration [36], and may also be affected by patients' hematocrit [37] and lipoprotein status [38, 39]. Whole blood is therefore the preferred matrix for therapeutic drug monitoring

of total CsA. In solid organ allografts Vd at steady state after intravenous administration has been reported to be between 3 to 5 L/kg [26].

1.4.5 Elimination/metabolism

Elimination of CsA is primarily via metabolism in the liver and the small intestine followed by excretion of metabolites in the bile (figure 3) [30]. Only 6% of administrated dose is eliminated by the kidney, with 0.1% excreted unchanged [26].

CsA is extensively metabolised to more than 30 metabolites by the cytochrome P-450 (CYP) 3A enzyme system [40]. CYP3A4 is the prominent enzyme in this subfamily [41], and accounts for about 80% of CsA metabolism [42]. Other isoenzymes, like CYP3A3 and CYP3A5, are also involved in the metabolism of CsA [43]. The importance and significance of the metabolites in terms of immunosuppressive activity and toxicity is not well-defined. However, there are works that indicate a correlation between blood concentrations of metabolites and nephrotoxicity, especially secondary metabolites like AM19 and AM1c9 [44, 45]. Furthermore, a study by Dai et al. demonstrated that CYP3A5 polymorphism has an impact on the formation of secondary metabolites. More AM19 and AM1c9 were formed with liver and kidney microsomes with a *CYP3A5*1/*3* genotype, compared to those with a *CYP3A5*3/*3* genotype, particularly in kidneys carrying the wild-type *CYP3A5*1/*1* [46].

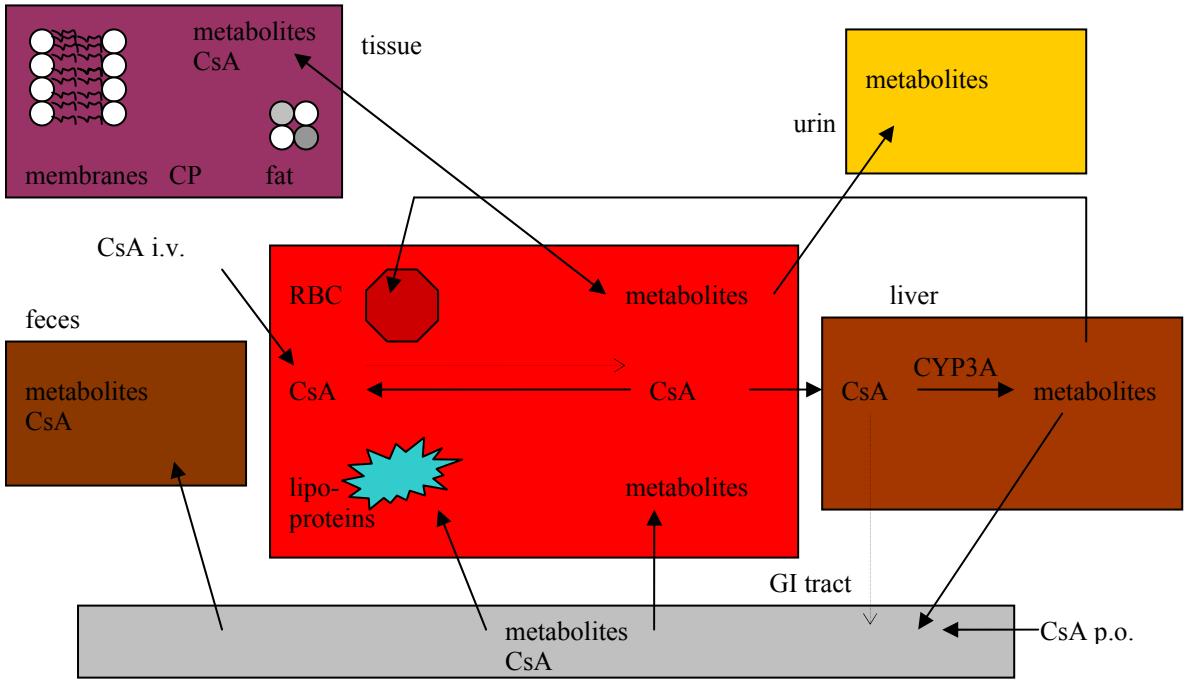

Figure 3: Overview of the distribution and elimination of CsA with metabolites.
CsA: ciclosporin A, **CP**: cyclophilin, **RBC**: red blood cells, **p.o**.: per oral, **i.v**.: intravenous.
Based on figure from Christians et al. [47].

1.4.6 P-glycoprotein (P-gp)

CsA is both a substrate and an inhibitor of the ATP-driven efflux pump P-glycoprotein (P-gp) [48]. P-gp is encoded by the *ABCB1* gene and is expressed in several locations in the body, including T-lymphocytes [49]. P-gp transports CsA out of T-lymphocytes, and the expression of P-gp could therefore affect its pharmacodynamic effect. An up-regulation of P-gp in T-lymphocytes after renal transplantation [50], and in CsA-resistant patients [51] has been demonstrated. Measurements of intracellular CsA concentrations in T-lymphocytes could therefore be an important factor with regards to efficacy.

P-gp is expressed in gut epitel cells, and some data suggest that the high unpredictability in CsA absorption found *in vivo* is associated with level of intestinal P-pg [52]. Variability in P-gp expression can therefore also be important with regards to bioavailability.

1.4.7 Therapeutic drug monitoring (TDM)

Historically, trough levels (C_0) were used to monitor CsA therapy. However, studies have shown that C_0 is a poor indicator of clinical outcome and total drug exposure [53]. AUC_{0-12} is a better predictor of outcome [54]. However, AUC_{0-12} can not be used in clinical practice because it is time consuming, expensive and inconvenient. Attention became focused on sampling during the first four hours (AUC_{0-4}), the absorption phase, where the variability is at its maximum. AUC_{0-4} was shown to correlate well with AUC_{0-12}, and was predictive for clinical outcome (both toxicity and rejection) [55]. It was further shown that the concentration 2 hours post dose (C_2) was the single point measurement that correlated best with AUC_{0-4} in renal transplant recipients [56]. Besides being a practical and convenient method in clinical settings, C_2 monitoring is considered to be a feasible TDM method today that also give lower acute rejections frequencies [57, 58]. However, the clinical benefit of C_0 over C_2 monitoring has still not been fully proven [59].

1.4.8 Pharmacokinetic population models of CsA in the literature

There are several published pharmacokinetic population models for CsA in renal transplant recipients using NONMEM in the literature. The choice of compartment model varies; one [60, 61]-, two- [62, 63] and three [64]- compartment models have been used to fit CsA whole blood concentrations. Both zero [62] and [60] first order kinetics are used to describe the absorption phase. A delay in the absorption of CsA is often observed. For the models that includes a delay function in the absorption phase both a lag-time parameter [62, 65] and Erlang distribution/gamma model [63, 66, 67] have been used. Some published models do not account for the delayed absorption [64, 68]. However, few of the population models have been externally validated.

1.5 AIMS

The purpose of this thesis was to develop a pharmacokinetic population model for CsA using NONMEM. The specific aims were:

1. Examine different compartment models with different absorptions profiles, in order to find the pharmacokinetic population model that describes the data best.
2. Screen for significant covariates that can reduce interindividual variability in the pharmacokinetic parameters.
3. Validate the final pharmacokinetic population model, with internal and external methods.

2 MATERIALS AND METHODS

2.1 PATIENTS

Twelve hour concentration profiles of CsA, performed within three months after transplantation, from 17 kidney transplant recipients were used in this thesis to develop a pharmacokinetic population model. These 17 patients participated in two different clinical trials; the MIMPARA study [69] and the SUPER-CsA study [70].

The MIMPARA study is an interaction-study including 14 renal transplant patients with stable renal function, of which 8 patients were treated with CsA. These 8 patients had one full twelve hour concentration profile performed. The SUPER-CsA study is a single centre prospective pilot study including 20 kidney transplant patients, all on a CsA-based immunosuppressive regimen. The patients were included within two weeks post transplant and followed for three months. 9 patients had one full twelve hour pharmacokinetic profile performed. The patients with full twelve hour concentration profiles for CsA performed from these two studies were used to develop a pharmacokinetic population model.

The main characteristics of the patients studied in this thesis are listed in table 2.

Table 2: Patient demographics.

Patient ID	CsA morning-dose (mg)	Sex (F/M)	Age (yrs)	Weight (kg)	Height (cm)	Serum creatinin (µmol/L) *	Steroid dose (mg)	Post trans-plantation period (weeks)	Diabetes
Super CsA study									
7	100	F	60	70	165	155	10	9.0	
8	225	M	59	90	185	95	20	4.1	
9	200	F	33	76	180	144	20	7.6	
10	150	M	35	68	185	184	15	4.6	
11	200	M	52	75	188	110	20	4.4	
12	225	M	67	97	181	133	15	4.3	
14	125	F	60	69	172	76	15	6.3	X
18	125	M	74	74	164	148	20	3.1	
19	350	M	52	80	176	142	20	2.1	X
MIM-PARA study									
30	150	M	25	92	182	131	10	9.3	
31	125	F	61	78	170	98	20	10.4	
32	150	M	59	91	179	103	20	9.0	X
33	175	F	68	78	156	92	20	3.0	X
34	125	F	69	86	164	109	15	6.9	
35	250	M	23	80	180	82	15	4.0	
36	125	M	52	86	189	127	20	3.0	
37	125	M	59	86	189	128	20	3.3	
Mean	172		53	81	177	121	17	5.6	
SD	64		15	9	10	29	4	2.6	

*Calculated with Nankivell formula; GFR (ml/min)= 6.7/SCr (mmol/L) + weight (kg)/4 – urea (mmol/L)/2 – 100/height^2 (m) + [35(man) eller 25(female)]
SD:standard deviation, **F**:femal, **M**:man

2.2 MODEL BUILDING

The pharmacokinetic population analyses were performed using the nonlinear mixed effect approach as implemented in NONMEM (version VI) [15]. Different compartment models were examined in order to find the best pharmacokinetic population model for CsA. One- two- and three-compartment models were tested, applying both first- and zero order absorption kinetics. The models were in addition tested with a lag-time parameter in the absorption phase. Erlang transit times for drug passage through the compartments were also examined, which can be used to describe lagged absorption processes. When using Erlang distribution as an absorption model, it is defined as the analytic solution for a linear chain of identical compartments placed upstream to the central compartment and connected by

identical rate constants (k_{tr}) [71]. OFV (objective function value) in NONMEM was used to optimize the number of lag-compartments (a) in the Erlang model (figure 4).

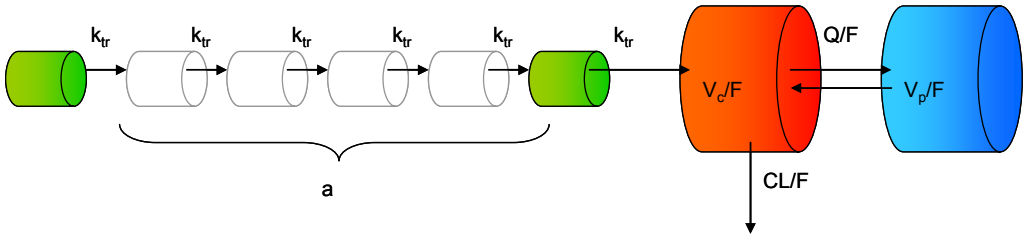

Figure 4: Erlang transit times for drug passage.

The models were parameterized in terms of volume of distribution (V_d) and clearance (CL), with an absorption rate constant (k_a). V_d and CL were represented as ratio to the unknown bioavailability (F), since CsA was administered orally.

The interindividual variability was described by an exponential error model, while the residual error was modeled using additive, proportional or combined error models.

Comparison between the tested models was based on:

- *Objective function value (OFV)*

The change in OFV was used to compare different models tested. If a new tested model produces a decrease in OFV ≥ 3.84, the new model gives a significant ($p<0.05$) better fit of the observed data.

- *Goodness-of-Fit (GOF):*

GOF plots give a basic internal evaluation of a model. Potential bias or problems in the structural model and/or the random effects models can be detected. GOF plots that were evaluated were ratio population predictions (PRED)/observation (OBS) versus time, ratio individual population predictions (IPRED)/observation (OBS) versus time, OBS versus PRED and IPRED, population residuals (RES) and population weighted residuals (WRES) versus PRED and WRES versus time, PRED and IPRED.

- *Parameters estimates and variability*

The likelihood of the estimated parameter values and the magnitude of interindividual variability and residual error were considered.

The interpatient variability in the pharmacokinetic parameters was estimated by calculating %CV (coefficient of variation). When using an exponential error model for the variability, %CV is determined by taking the square root of the eta value for that parameter and multiplying by 100. The intrapatient variability was also estimated by calculating %CV for the proportional error model, but given as absolute variability when using an additive error model. Additive variability is calculated by taking the square root of the eta value for that parameter.

2.3 COVARIATE ANALYSIS

The analysis for influence of covariates on the pharmacokinetic parameters was performed in accordance with traditional methods [72-74]. First graphical analyses were conducted to study the relationship between each parameter and covariate according to the method described by Maitre et al. [74]. The demographic parameters of interest (x-axis) were plotted against the individual estimated pharmacokinetic parameters (y-axis). The individual estimated pharmacokinetic parameters were obtained using the *"posthoc"* subroutine in NONMEM, and the statistic program R was used to create the scatter plots. From the scatter plots covariates that correlate with the pharmacokinetic parameters can be identified. Correlations seen in the scatter plots have the possibility of being clinically relevant, and were tested in the next step. Weak correlations in the scatter plots are probably not applicable for this model, and were not tested any further.

The influence of 7 cofactors was studied: weight (kg), creatinine clearance (mL/min), age (years), height (cm), gender, post-transplantation period (weeks) and steroid dose at the pharmacokinetic day (mg/day).

The demographic factors showing a correlation with a pharmacokinetic parameter were further tested using the "forward inclusion-backward elimination" method [72-74]. Each of the covariates found in the first step was introduced separately into the structural model to

asses its impact on the pharmacokinetic parameters. Covariates were modeled as being both proportional and linear to the typical parameter value. The covariates centred on the mean covariate value were also tested, exemplified by the typical value of distribution volume (TVV) and bodyweight (WT):

Proportional model: $$TVV = \theta_1 * WT * EXP(ETA(1)) \quad (5)$$

Linear model (mean centred): $$TVV = [\theta_1 + \theta_2 * (WT - medianWT)] * EXP(ETA(1)) \quad (6)$$

The likelihood ratio was used to test the effect of each covariate on the pharmacokinetic parameters in this next step. A covariate was selected significant if it produced a decrease in OFV $\geq$ 3.84 ($p<0.05$) from the covariate free model [73]. All the significant covariates were then added simultaneously into the covariate free model. The OFV for this model, including all the statistically significant covariate-parameters relationship, was noted. Thereafter, in a backward deletion strategy, each covariate was taken out of the model independently from the full model. An increase in OFV $\geq$ 6.6 ($p<0.01$) was required to consider the covariate as significant and to keep it in the model [73]. Finally, all the significant covariates were introduced into a final model.

It is well known that some patients show a very slow absorption profile, and the relationship between slow absorption profile and the presence of diabetes was tested [75]. Therefore, slow absorption profile was first considered to be a binary covariate, using a FLAG function in NONMEM. The patients with diabetes were "flaged", and NONMEM estimated a separate absorption constant (k_a) for these patients. The change in OFV and GOF plots were used to evaluate whether this produced a better model.

An another method was also used in order to account for two different absorption constants, without taking the conclusion the reason was diabetes. This was done by using the mixture function in NONMEM [76]. This means that NONMEM will divide the population into the number of subpopulation decided beforehand, without deciding which patients are in which population in advance. Here two subpopulations with two different absorption constants were tested. In the same procedure, the change in OFV and GOF plots were used to evaluate whether this improved the model.

2.4 VALIDATION

2.4.1 Posterior predictive check

A posterior predictive check method [77] was chosen as an initial validation procedure. With this approach the compatibility of the data and model is assessed by comparing simulated concentrations with observed concentrations. Simulated concentrations were estimated via the simulation function in NONMEM. A dataset with significant covariates, doses and time measurements, but without the observed concentrations, was created. The estimation command in NONMEM was replaced by a simulation command ($SIMULATION), and the thetas, omegas and sigmas were fixed to the estimates from the final model. 100 simulations were performed.

For each subject in each simulation, C_{max}, C_{trough} and AUC_{0-12} were calculated and compared with C_{max}, C_{trough} and AUC_{0-12} from the observed data. The mean values of the observed data were compared to 95% confidence interval (CI) limits from the simulated data. Paired statistic tests using SPSS were performed to determine whether the observed and simulated mean values were significant different.

2.4.2 Jackknife estimation

A Jackknife run was performed in order to find the confidence interval (CI) of the pharmacokinetic parameters. Each patient was in order excluded form the data set, which then gave 17 Jackknife datasets. These Jackknife datasets were examined in NONMEM using the final pharmacokinetic population model, producing a new set of estimates for the pharmacokinetic parameters. The pharmacokinetic estimates from the Jackknife datasets were tested for normality in SPSS, and a 95% CI were then calculated.

A Jackknife run will also identify individuals that have large influence on the estimation of the values of the parameters.

2.4.3 Data splitting

A data-splitting method was applied to confirm the robustness of the final model, and to determine the contribution of data from individuals in a subset group [78]. The full data set were divided into 10 subsets randomly. Each subset contained data from approximately 90% of the patients, and were examined in NONMEM using the final model. The 10 subset groups are presented in table 3.

Table 3: Subset groups.

Group	Patient(s) excluded	
1	8	
2	30	
3	36	
4	11	19
5	7	9
6	10	12
7	14	31
8	35	37
9	18	33
10	32	34

The parameter estimates determined from the subset analyses were compared in terms of the SD's of the parameters in the full dataset.

The OFV was also calculated by another NONMEM run for the full data set, but with the parameter estimates fixed at the estimates from the subset analyses. The OFVs obtained in this step were compared with the OFV from the full data set. 95% CI for the absolute difference in OFV is achieved if the absolute difference of these values from that of the final model is ≤ 3.84.

2.4.3.1 Predictive performance

The NONMEM estimates from each of the 10 subsets were used to predict CsA concentrations in the remaining 10% of the patients' data. 10 control files with initial estimates of theta, omega and sigma replaced by the estimates from the 10 subsets were created. The individual concentrations were estimated using the "*posthoc"* subroutine and with the $ESTIMATION command set to MAXEVAL = 0, which means that the estimation step will be omitted. A dataset with significant covariates and doses was created. The predictive performance was tested without any concentration measurements provided in the dataset, with one concentration at time 0 and 2 hours post-dose provided, with two concentrations at time 0 and 2 hours post-dose and time 1 and 2 hours post-dose provided and three blood samples at time 0, 1 and 2 hours post-dose and 0, 1 and 3 hours post-dose provided. The choices of time measurements was based on empiricism and the fact that $AUC_{0\text{-}4}$ is a good predictor for clinical outcome [55].

Estimated $AUC_{0\text{-}12}$ at the different time measurements given were compared with observed $AUC_{0\text{-}12}$, calculated using the linear-trapezoidal method. To evaluate predictive performance, the mean percentage prediction error (%MPE) and the mean precentage absolute prediction error (%MAPE) were calculated.

$$mpe\,(\%) = \frac{1}{N}\sum_{i=1}^{N}\frac{predicted\ value - observed\ value}{observed\ value}\times 100\% \qquad (7)$$

$$mape\,(\%) = \frac{1}{N}\sum_{i=1}^{N}\frac{\left|predicted\ value - observed\ value\right|}{observed\ value}\times 100\% \qquad (8)$$

Bias is estimated by mean prediction error (MPE) and the precision of the predictions is estimated by the mean absolute prediction error (MAPE).

2.4.4 External validation with Bayesian procedure

The Bayesian approach was applied to an external group of 10 kidney transplant patients. These new patients participated in a different study were the pharmacokinetics in elderly were examined [79]. The main characteristics of the patients in the external group are presented in table 4.

Table 4: Patient demographics in the external group.

Patient ID	**CsA morning -dose (mg)**	**Sex (F/M)**	**Age (yrs)**
A	225	M	28
B	200	M	67
C	275	M	29
D	175	F	55
E	150	M	78
F	225	M	63
G	175	M	64
H	125	F	73
I	300	M	48
J	125	M	75
Mean	198		58
SD	59		18

SD:standard deviation**, F:**femal**, M:**man

A MAP (maximum a posteriori) Bayesian estimator using the same time measurements as in the predictive check of the data splitting analyses were tested. The final pharmacokinetic population model was used to obtain Bayesian individual estimates of the pharmacokinetic parameters in the external validation set. Bayesian estimation was performed using the "*posthoc"* subroutine and with the $ESTIMATION command set to MAXEVAL = 0.

Predictive performance was evaluated in same procedure as explained in section 2.4.3.1.

2.5 NON-POPULATION ANALYSES

A non-compartmental analysis of the dataset was first performed. This was done by manual calculation in Excel. In addition a pharmacokinetic modeling analysis of the dataset using WinNonlin was performed. WinNonlin is a tool for nonlinear modeling. A 2-compartment model with first order absorption and a lag-time was chosen from the library in WinNonlin to fit the data.

This was done in order to test for significant different estimates of CL and V_d between non-compartment analysis, simple pharmacokinetic modeling and pharmacokinetic population modeling.

2.6 STATISTICS

When testing different models in NONMEM, the models were considered statistic different if $p < 0.05$ (corresponding to OFV ≥ 3.84).

Statistic analyses were performed using SPSS for Windows (version 12). Normality was first assessed to determine which statistic analysis to apply. In the predictive check analysis, student's t-test was used to assess differences between observed and simulated values for $AUC_{0\text{-}12}$ and C_{max} (normally distributed), and Wilcoxen matched pairs signed ranks test was used to assess differences between observed and simulated values for C_{min} (not normally distributed) [80]. When testing for significant differences in the estimation of CL/F and V_d/F between non-compartment calculations, WinNonlin and NONMEM, one-way repeated measures ANOVA test was used to asses differences in the estimation of CL/F (normally distributed), and Friedman Test was used to asses differences in the estimation of V_d/F (not normally distributed) [80].

3 RESULTS

3.1 DIFFERENT COMPARTMENT MODELS WITH DIFFERENT ABSORPTION PROFILES

The 2-compartment model with Erlang distribution in the absorption phase had the lowest OFV of all models tested (table 5). The residual variability was about the same for the 2-compartment model with Erlang distribution, the 2-compartment model with lag-time and the 3-compartment model with lag-time.

Table 5: Comparison of different covariate free models tested for modeling CsA pharmacokinetics based on Objective Function Value (OFV) and residual variability.

Model tested	OFV	Residual variability (Proportional/Additive)
1-compartment with first order absorption	2584	39.50%
1-compartment with first order absorption and a lag-time	2510	30.58% / 55.86 µg/L
2-compartment with zero order absorption	2571	37.95%
2-compartment model with first order absorption	2488	31.00%
2-compartment model with first order absorption and a lag-time	2282	13.08% / 37.42 µg/L
3-compartment model with first order absorption	2293	13.67% / 35.50 µg/L
2-compartment model with Erlang distribution as an absorption process	2280	13.53% / 37.55 µg/L

The CL1/F estimates were similar between the different models, however the distribution volumes and absorption constants considerably differed between them (table 6).

Table 6: Pharmacokinetic parameter estimates in the different models tested.

Model	CL1/F (L/h)	V1/F (L)	CL2/F (L/h)	V2/F (L)	CL3/F (L/h)	V3/F (L)	K_a (h^{-1})	Lagtime (hrs)
1-compartment	20.6	117					1.92	
1-compartment with lag-time	20.2	113					5.55	0.438
2-compartment 0. order absorption	16.3	1.00	64.1	78.0			0.320	0.300
2-compartment	22.0	47.7	17.8	991			0.802	
2-compartment with lag-time	21.4	27.4	22.8	337			1.03	0.454
2-compartment with Erlang absorption	21.8	58.8	23.1	245			7.90*	
3-compartment with lag-time	21.4	50.9	10.5	32.3	12.8	5630	1.92	0.451

*$\mathbf{k_{tr}}$ (transfer rate constant between the sequential compartments) in the Erlang model
CL1/F = apparent clearance, **V1/F** = volume of the central compartment, **CL2/F** = intercompartment clearance 1, **V2/F** = volume of peripheral compartment 1, **CL3/F** = intercompartment clearance 2, **V3/F** = volume of peripheral compartment 2, $\mathbf{K_a}$ = absorption rate constant

For the 3 models with lowest OFV (2-compartment model with lag-time, 3-compartment model with lag-time and 2-compartment model with Erlang distribution) the predicted concentrations correlated generally well with the observed concentrations, as seen in figure 5. However, in the 2-compartment model with Erlang distribution in the absorption phase the parameter estimates were highly robust compared with the 2-compartment model with lag-time and the 3-compartment model with lag-time. For these two models, the parameters were very sensitive for initial estimates. In the other compartment models examined, NONMEM was not able to predict the highest concentrations (figure 5). Moreover, the absorption phase was poorly described without accounting for a delay in absorption, as done with a lag-time parameter or Erlang transit time for drug passage.

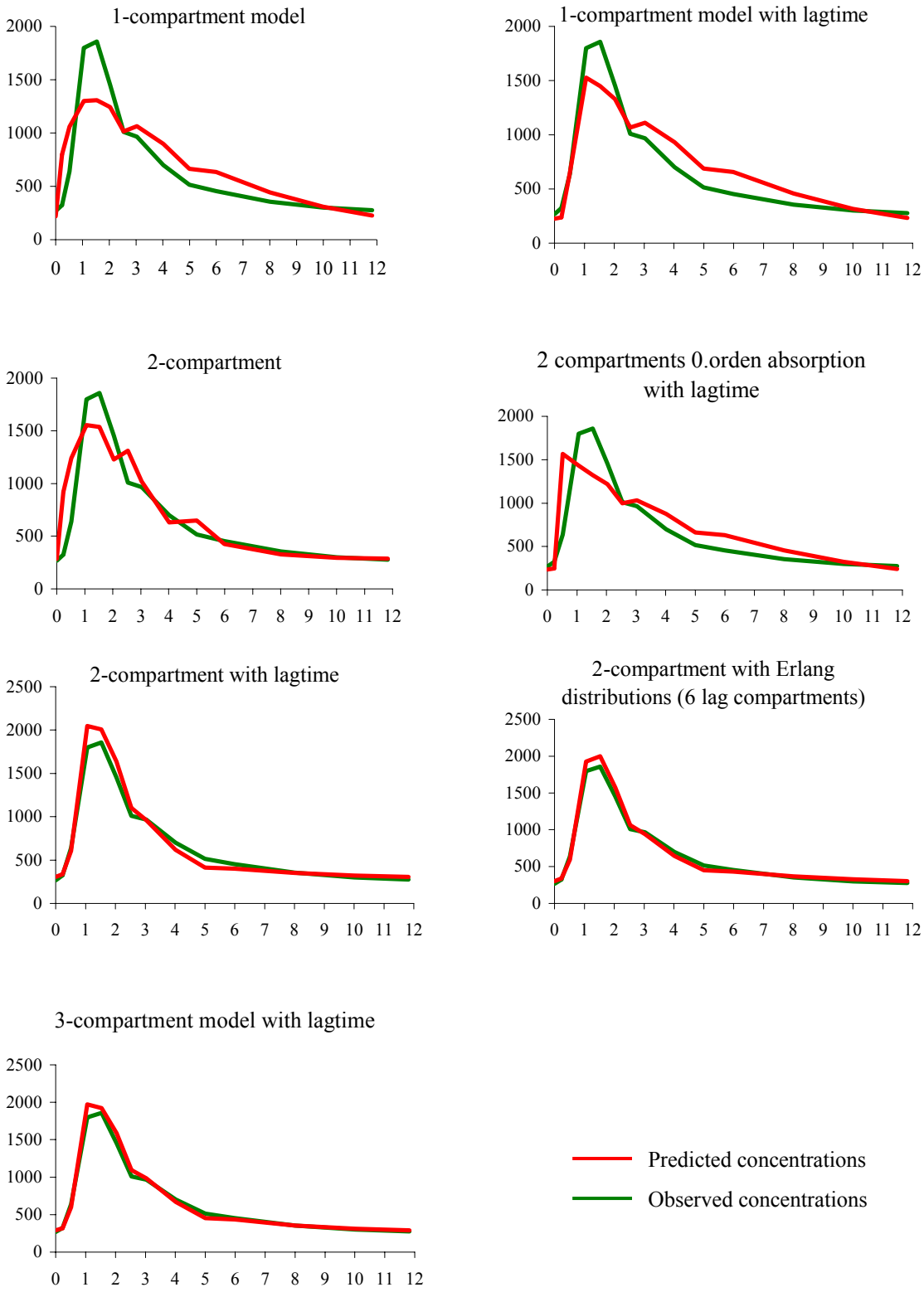

Figure 5: Concentration-time curves for the different compartment models tested.

Concentration (µg/L) is given at the y-axis and time (hrs) is given at the x-axis. Red lines are the concentrations predicted by NONMEM, and the green lines are the measured concentrations. The type of compartment model is indicated over the graph.

For the Erlang model, the optimal number of sequential compartments placed upstream to the central compartment was six. Including one more sequential compartment did not lead to significant change in OFV (table 7).

Table 7: Results for 2-compartment model with Erlang distribution as an absorption process with increasing number of sequential compartments.

	CL /F (L/h)	V_c/F (L)	V_P/F (L)	Q/F (L/h)	K_{tr} (h^{-1})	OFV
1 LAG	22.2	34.5	237	22.6	1.45	2402.50
2 LAG	21.8	22.9	150	28.3	2.11	2338.90
3 LAG	21.7	40.8	151	27.5	3.62	2310.53
4 LAG	21.6	49.3	179	25.4	5.05	2293.03
5 LAG	21.8	55.2	209	24.1	6.45	2284.06
6 LAG	21.8	58.8	245	23.1	7.86	2280.00
7 LAG	21.8	61.5	284	22.3	9.27	2279.14

LAG = number of sequential compartments placed upstream to the central compartment **k_{tr} =** transfer rate constant between the sequential compartments**, CL/F =** apparent clearance**, V_C/F =** volume of the central compartment**, Q/F =** intercompartment clearance**, V_P/F =** volume of peripheral compartment **F =** bioavailability

3.2 COVARIATE ANALYSIS

3.2.1 Graphical analysis

From the graphical analyses conducted, weight, age and creatinine clearance tended to correlate with some of the pharmacokinetic parameters (figure 6). These covariates were therefore tested further for their significance with the inclusion-deletion method. The other covariates tested had low coefficient of determination values (r^2).

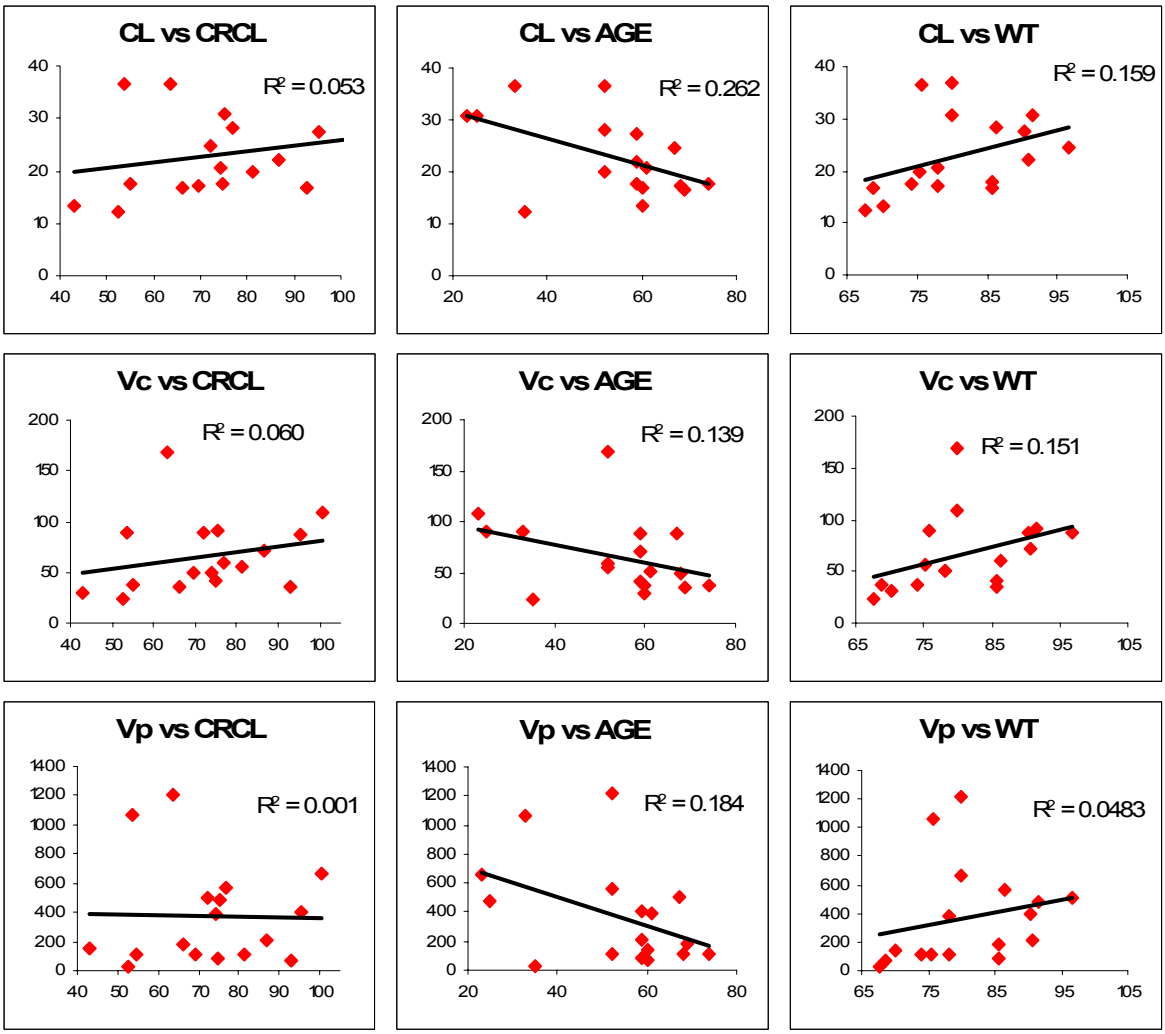

Figure 6: Graphical analysis.

An extract of graphs for testing correlations between pharmacokinetic parameters and covariates.

V_C = volume of the central compartment, **V_P**= volume of peripheral compartment, **CL** = apparent clearance, **WT**= weight, **CRCL** = creatinine clearance.

3.2.2 Inclusion-deletion method

From the inclusion step, with weight as a covariate on V_C/F a reduction in OFV of 1.58 was achived, however the OFV value did not change when modeling weight as a covariate on V_P/F (table 8). A slightly reduction in OFV was also seen when modeling weight as a covariate on Q/F (ΔOFV = 0.82). Creatine clearance (CRCL) as a covariate on CL/F gave a reduction in OFV of 0.88, in addition to a reduction in OFV of 0.96 when modeling CRCL as a covariate on V_C/F. All these relationships were insignificant, and were therefore not tested further.

Age as a covariate of CL/F gave a reduction in OFV of 5.62 in the inclusion step, which is significant. The relationship was CL/F = TVCL – θ * AGE where TVCL is the typical value of clearance, and θ had a mean value of 0.116. The interindividual variability of clearance was slightly reduced from 32.5% to 29.8%. However, the interindividual variability of V_P/F was reduced from 110% to 95.6%. Since this was the only covariate that gave a statistically significant reduction in OFV by inclusion, the deletion step could not be performed.

The relationship between diabetes and slow absorption profile were tested using a flag function. Including a flag function in the model did not give a better fit of the CsA data. Both the GOF plots and OFV (ΔOFV = 0.7) were about the same as in the covariate free model. The estimated k_{tr} for diabetics were 7.84, compared with 7.87 in non-diabetics.

Including the mixture function for the absorption constant in the covariate-free model gave a significant reduction in OFV (ΔOFV = -5.5) and better GOF plots. However, NONMEM placed only one patient in the subpopulation with slower absorptions profile. The impact of having two different absorption constants was therefore considered not to be clinically relevant.

None of the other covariates induced statistically significant decrease in OFV, as can be seen from table 8.

Table 8: Changes in OFV due to inclusion of covariates; the inclusion step.

	Δ OFV				
	CL/F	**V_C/F**	**V_P/F**	**Q/F**	**K_{tr}**
WT	0.01	-1.58	0.00	-0.82	
CRCL	-0.88	-0.96	0.00	0.01	
AGE	-5.62	0.01	0.00	0.00	
MIXTURE					-4.44

WT = weight, **CRCL** = creatinine clearance, **CL/F** = apparent clearance, **V_C/F** = volume of the central compartment, **V_P/F** = volume of peripheral compartment, **Q/F** = intercompartment clearance, **k_{tr}** = transfer rate constant between the sequential compartments, **F** = bioavailability.

3.3 THE BEST PHARMACOKINETIC POPULATION MODEL

The best pharmacokinetic population model found for the CsA dataset was a 2-compartment model with Erlang distribution in the absorptions phase and age as a covariate for clearance.

3.3.1 Parameter estimates with variability

The mean values of population parameters and the interindividual variability obtained in the 2-compartment model with Erlang distribution are listed in table 9.

Table 9: Pharmacokinetic parameters and interindividual variability in the final model.

Parameter	K_{tr} (h^{-1})	CL/F (L/h)	V_C/F (L)	Q/F (L/h)	V_P/F (L)	Covariate
Mean	7.84	28.1	58.8	23.1	215	0.116
95% CI*	7.78-7.91	27.5-28.7	57.6-59.5	22.9-23.3	205-226	0.109-0.126
Interindividual variability (%CV)	24.2	29.8	52.1	14.5	95.6	

*Calculated in section 3.4.2
CI = confidence interval, **CV** = coefficient of variation, $\mathbf{k_{tr}}$ = transfer rate constant between the sequential compartments, **CL/F** = apparent clearance, $\mathbf{V_C/F}$ = volume of the central compartment, **Q/F** = intercompartment clearance,$\mathbf{V_P/F}$ = volume of peripheral compartment, **F** = bioavailability.

The residual error of the model (table 5) was 13.5% (proportional error model) and 37.6 µg/L (additive error model).

3.3.2 Goodness-of-fit (GOF) plots

The goodness-of-fit (GOF) plots presented in figure 7 showed no indication of model misspecification. The plots of ratio OBS/PRED versus time and ratio OBS/IPRED versus time showed no relevant bias over or under the value of 1, which is the value if PRED or IPRED is identical with OBS. The distribution of WRES as a function of sampling times and ID was homogeneous, and WRES were in an acceptable range. One WRES was >5, which can be an indication of an outlier. Moreover, the scatter plots of PRED and IPRED versus OBS did not show bias and the plots showed good correlations. The coefficient of determination (r^2) was high for IPRED (r^2=0.95).

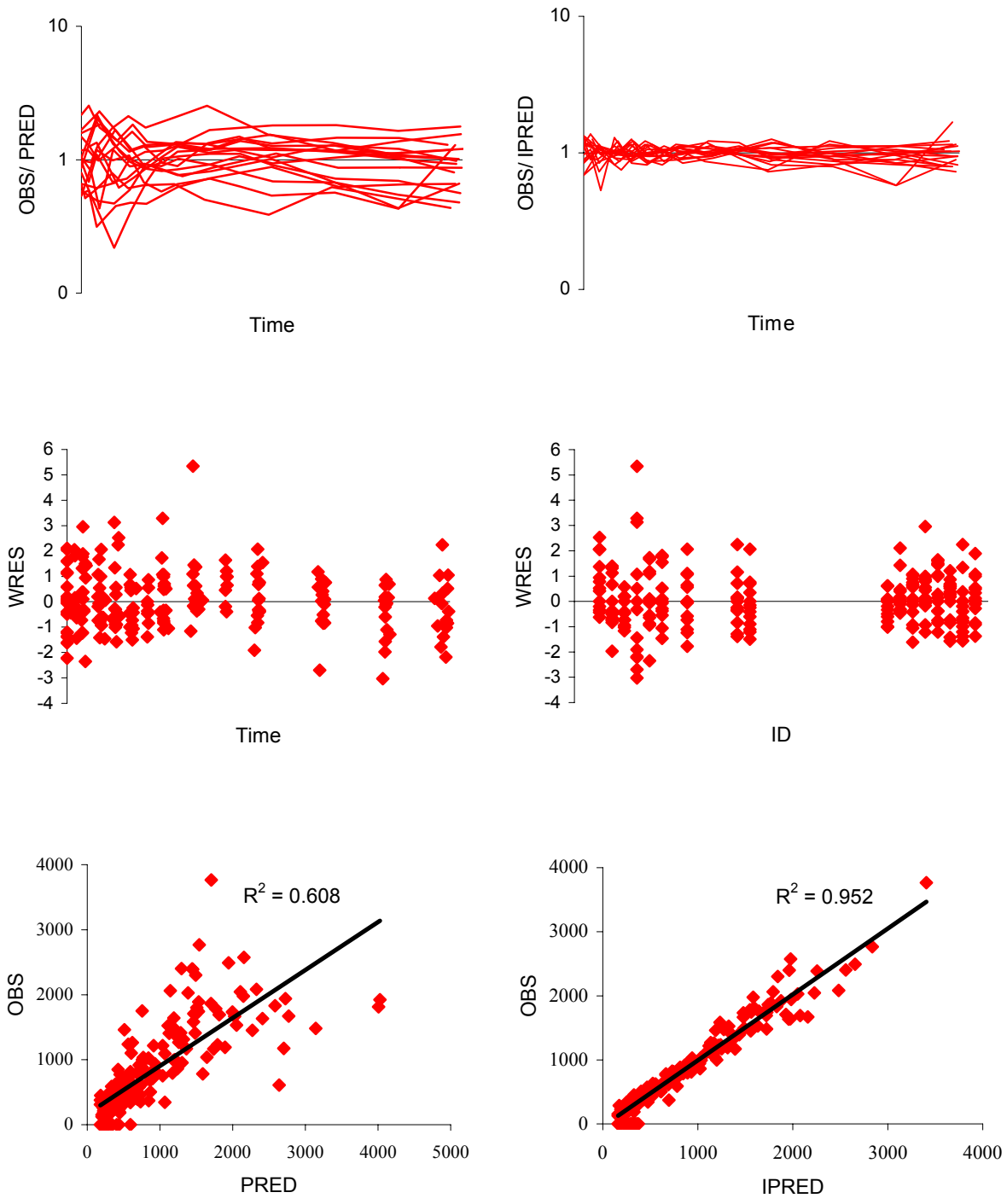

Figure 7: GOF plots for the 2-compartment model with Erlang distribution.

OBS = observed concentrations, **PRED** = predicted concentrations, **IPRED** = individual predicted concentrations, **WRES** = weighted residual error, **ID** = patient number.

3.3.3 Individual fits

The final population model described the pharmacokinetic data of CsA well, as seen in the individual plots (appendix). Two of the best and worst fits are shown in figure 8.

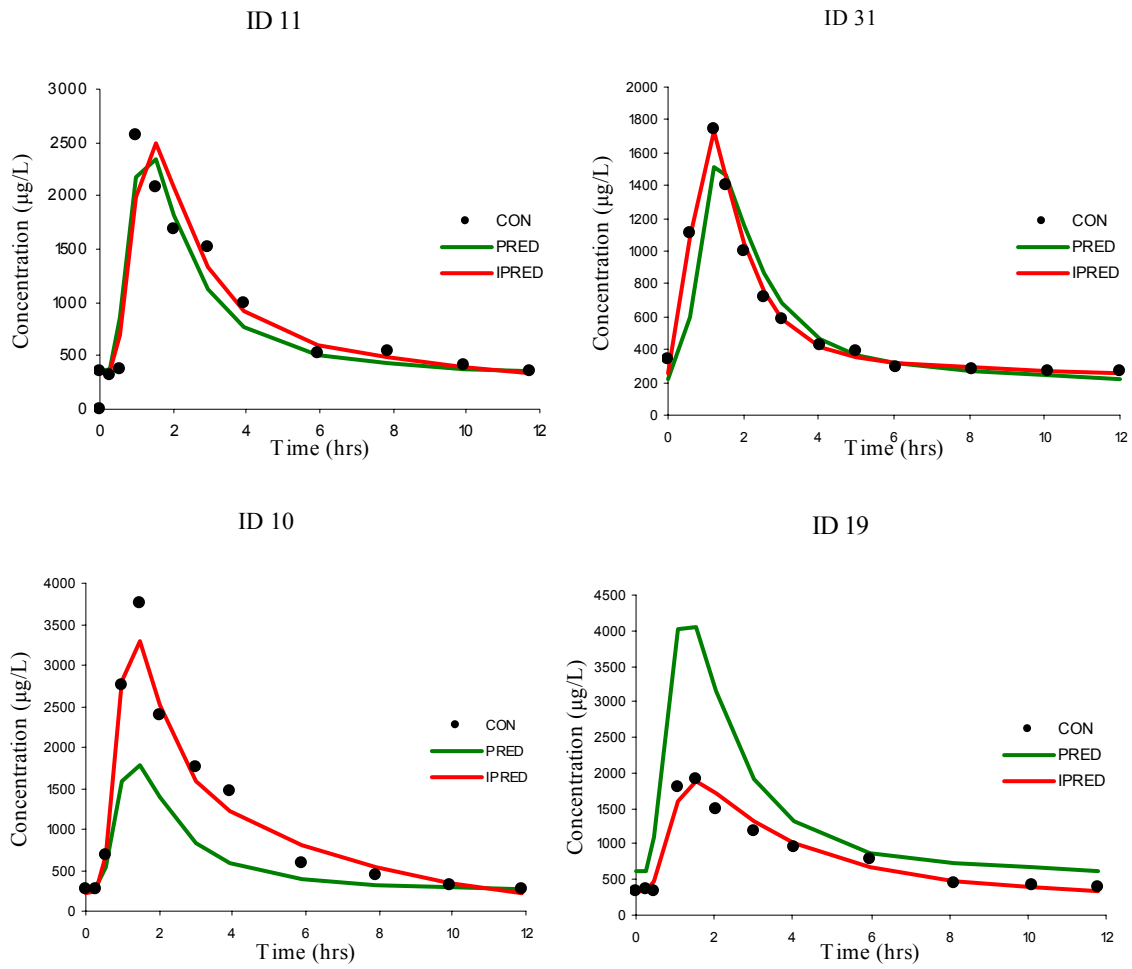

Figure 8: The best and worst fits.

ID 11 and 31 represents the best fits, and ID 10 and 19 represents the worst fits. The circles are the concentrations observed (**CON**), the green lines are the predicted concentrations (**PRED**) and the red lines are the individual predicted concentrations (**IPRED**).

3.3.4 Control file

The control file for the final pharmacokinetic population model is presented in figure 9 below.

```
$PROB Erlang distribution 6 lag; States the problem being solved

$DATA Inputfil.txt; Name of dataset

$INPUT ID AMT RATE TIME CON=DV MDV SS II CMT AGE ; Identifies columns in dataset
;ID=patient ID, AMT= amount of drug administered (µg), RATE: route of administration, TIME=
time of concentration measurement (hours), CON=concentrations measured (µg/L), DV=dependent
value, MDV=missing data variable, SS=steady state, II:dose interval, CMT: defines in which
compartment DV is observed, AGE=age of patient (years)

$SUBROUTINE ADVAN5 SS5; Set up differential equation mode

$MODEL COMP=(DEPOT,DEFDOSE) ; Defines the number of compartments
   COMP=(DELA1)
   COMP=(DELA2)
   COMP=(DELA3)
   COMP=(DELA4)
   COMP=(DELA5)
   COMP=(DELA6)          ; "Erlang" compartments
   COMP=(CENTRAL,DEFOBS) ; Central compartment
   COMP=(PERIPH)         ; Pheripheral compartment

$PK ; Define basic pharmacokinetic parameters
  K12=THETA(1)*EXP(ETA(1)) ; Rate constant between the delay compartments
  K23=K12
  K34=K12
  K45=K12
  K56=K12
  K67=K12
  K78=K12

  CLTV=THETA(2)-THETA(6)*AGE ; Clearance depends on age
  V8TV=THETA(3)
  V9TV=THETA(4)
  QTV=THETA(5)

  CL=CLTV*EXP(ETA(2)) ; Clearance
  V8=V8TV*EXP(ETA(3)) ; Central volume of distribution
  V9=V9TV*EXP(ETA(4)) ; Pheriperal volume of distribution
  Q=QTV*EXP(ETA(5)) ; Intercompartment clearance

  K80=CL/V8 ; Micro constant between central compartment and out of the system
  K89=Q/V8  ; Micro constant between central and peripheral compartment
  K98=Q/V9  ; Micro constant between peripheral and central compartment

  S8=V8     ; Scale for central compartment
```

```
$ERROR
  IPRED=F
  Y=F+F*ERR(1)+ERR(2) ; Additive and proportional residual error model

$THETA ;
 (1,7.8) ; K12 (B)
 (10,22) ; Q/F
 (10,58) ; V8
 (10,244) ; V9
 (10,23) ; CL/F
 (0.001,0.05) ; age effect

$OMEGA ; Variance of interindividual variability
0.06 ; K12

$OMEGA BLOCK(4) ; Variance of interindividual variability
0.1                ;CL
0.02  0.1            ;V8
0.02  0.02  0.1        ;V9
0.02  0.02  0.02 0.1   ;Q

$SIGMA 0.1 ; Variance of intraindividual variability, proportional error model
$SIGMA 25 ; Variance of intraindividual variability additive error model

$ESTIMATION SIG=3 MAX=9999 PRINT=1 METHOD=1 INTER POSTHOC
;SIG=number of significant digits in the final parameter estimates
;MAX= maximal number of iterations (function evalutions) before NONMEM gives up
;PRINT=determines how often summaries of iterations are printed
;METHOD: 0 when the FO estimation method is used, and 1 when the FOCE method is used.
;INTER: required when using the FOCE method;
;POSTHOC: optaines individual estimates of the parameters

$COVARIANCE ; Requests that the covariance step be implemented (optional)

$TABLE ID TIME DV IPRED
   NOPRINT ONEHEADER FILE=table.txt; Prepare an output table of results

$TABLE ID V8 V9 CL Q WT CRCL AGE SEX HT TXT STER ETA1 ETA2 ETA3 ETA4
   FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
   ; Prepare an output table of results
```

Figure 9: Control file for the 2-compartment model with Erlang distribution to describe the absorption phase. Explanations are given after semicolon, and will not be recognised by NONMEM.

3.4 MODEL VALIDATION

3.4.1 Posterior predictive check

The 95% confidence interval (CI) of C_{max}, C_{trough} and AUC_{0-12} from the posterior predictive check contained the true observations. Also paired statistic tests performed using SPSS showed no significant distinguish ($p>0.18$) between observed and simulated values of C_{max}, C_{trough} and AUC_{0-12} (table 10).

Table 10: Results from posterior predictive check.

	Observed values (mean)	**Simulated values (95% CI)**	**P-value**
AUC_{0-12} (µg/L*h)	7671	6867-9567	0.435
C_{trough} (µg/L)	288	278-376	0.177
C_{max} (µg/L)	2090	1735-2477	0.950

3.4.2 Jackknife

The 95% CI for the pharmacokinetic parameters were calculated from the Jackknife estimates, and are presented in table 11. No individuals showed any significant influence on the pharmacokinetic parameters (table 11).

Table 11: Pharmacokinetic parameter estimates from the Jackknife run of the 2-compartment model with Erlang distribution.

		Patient excluded								
Covariate	**Final model**	**7**	**8**	**9**	**10**	**11**	**12**	**14**	**18**	**19**
k_{tr}	7.84	7.86	7.74	7.81	7.89	7.89	7.65	7.87	7.91	7.88
CL/F	28.1	28.3	28.0	25.6	31.3	28.1	26.7	28.6	29.8	27.2
V_C/F	58.5	60.2	56.6	56.8	62.0	58.2	55.6	60.7	60.1	55.0
V_p/F	215	188	202	203	233	251	191	242	216	202
Q/F	23.1	23.4	23.4	22.8	23.6	23.3	23.2	23.2	23.7	22.8
AGE effect on CL	0.116	0.109	0.121	0.082	0.158	0.116	0.097	0.120	0.146	0.113

	Patient excluded							
	30	**31**	**32**	**33**	**34**	**35**	**36**	**37**
k_{tr}	7.98	7.74	7.95	7.66	7.77	8.19	7.81	7.73
CL/F	27.2	28.2	27.9	27.7	28.4	29.2	27.4	28.5
V_C/F	57.0	59.3	58.3	58.1	61.4	56.7	59.5	60.0
V_p/F	203	199	246	227	230	188	203	245
Q/F	23.4	22.6	22.9	23.6	22.3	22.8	22.6	23.2
AGE effect on CL	0.107	0.119	0.114	0.106	0.117	0.140	0.111	0.119

			95% CI	
	Mean	**SD**	**lower**	**upper**
k_{tr}	7.84	0.131	7.78	7.91
CL/F	28.1	1.26	27.5	28.7
V_C/F	58.6	2.06	57.6	59.5
V_p/F	216	21.8	205	226
Q/F	23.1	0.407	22.9	23.3
AGE effect on CL	0.117	0.0178	0.109	0.126

k_{tr} = transfer rate constant between the sequential compartments, **CL/F** = apparent clearance**, V_C/F** = volume of the central compartment, **V_P/F** = volume of peripheral compartment **Q/F** = intercompartment clearance, **F** = bioavailability

3.4.3 Data splitting

Figure 10 shows the parameter estimates for the full data set and for the 10 different subsets. All pharmacokinetic parameters estimates in the subsets, with exception of four estimates, were in the range of ± 2 SD of the final estimates. Moreover, the majority of the values were in the range of ± 1 SD.

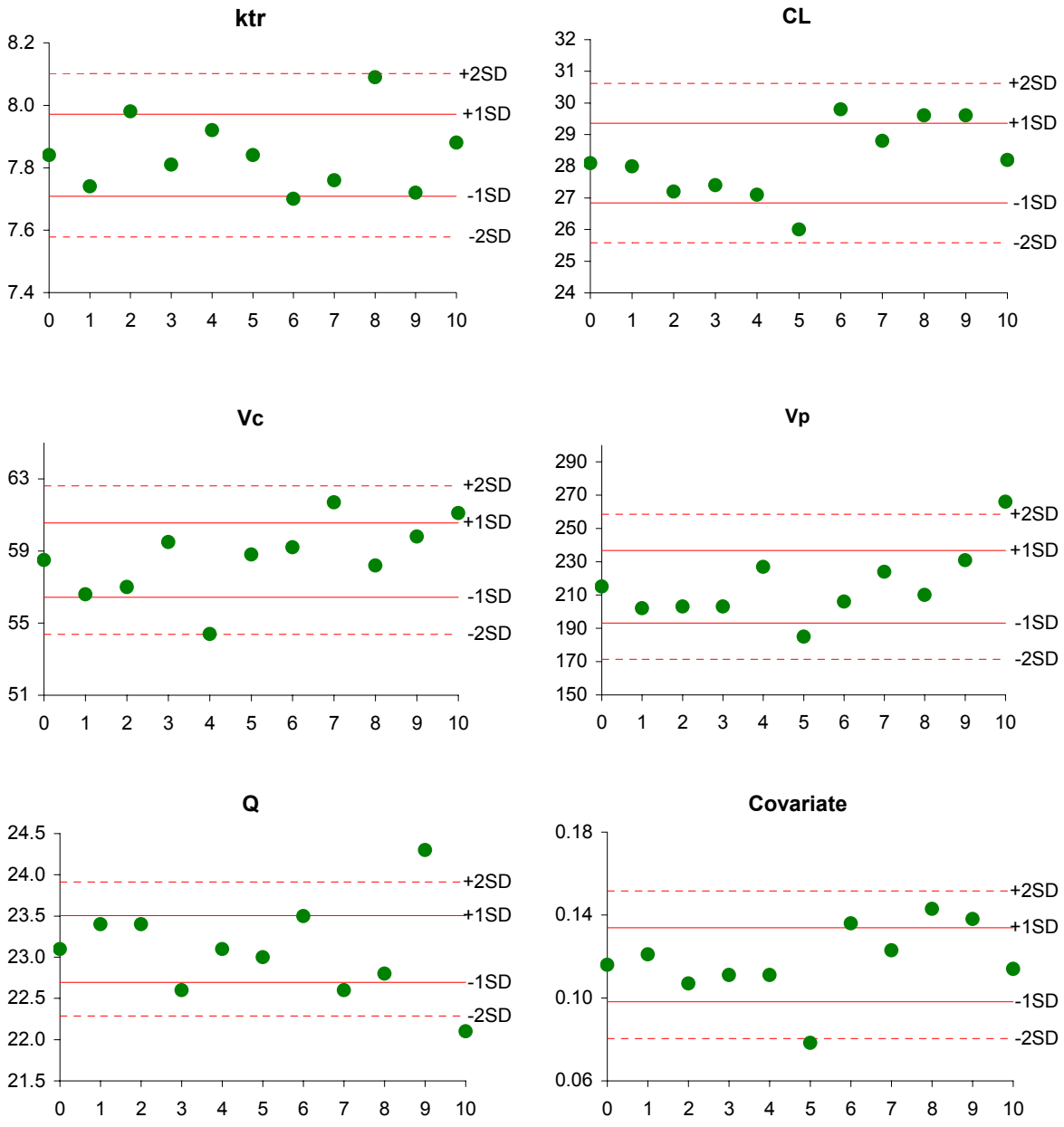

Figure 10: Data splitting.

Pharmacokinetic parameter estimates for the full dataset and the 10 subsets. Plots on the x-axis at value 0 are from the full dataset, and plots on the x-axis at values 1 to 10 are from the 10 subsets. The standard deviations (SDs) were calculated based on the Jackknife estimates.

The OFVs obtained by another NONMEM run for the full data set fixing the parameter estimates for the 10 subsets were in range from 2274.5 to 2275.4, which gives a non-significant absolute difference from the final model (ΔOFV = 0.9).

3.4.3.1 Predictive performance (internal)

In order to examine predictive performance of the final model, the NONMEM estimates from each of the 10 subsets were used to predict the concentration profiles in the remaining 10% of patients' data. Individual $AUC_{0\text{-}12}$ were calculated from the plasma concentration profiles using limited time measurements, and the result of bias (MPE) and prediction (MAPE) in each subset are presented in table 12. Without any information of the concentrations provided in the dataset, the mean absolute prediction error (MAPE) was 18.6%, which was due to over-prediction of the observed concentrations (+8.5% bias). When using only one time sample, C_0 or C_2, the prediction was reduced to about 10%. As expected, the prediction was better with two or three concentration measurements provided in the dataset.

Table 12: Predictive performance in the subset groups.

Subset	No info	C_0	C_2	C_0+C_2	C_1+C_2	C_0+C_1+C_2	C_0+C_1+C_3
1	33.9	27.7	22.1	25.1	16.5	21.7	11.6
2	24.3	9.2	22.5	11.9	15.0	4.7	7.2
3	34.2	4.9	4.0	-4.9	-0.1	-5.0	-2.3
4	32.6	13.9	-0.4	3.0	5.3	2.2	16.4
5	10.5	17.0	0.8	10.8	2.4	11.5	7.2
6	-8.2	-10.8	-7.8	-4.7	-0.4	-0.2	2.8
7	-12.2	2.4	-11.4	0.05	-7.2	4.5	5.6
8	-1.1	-1.7	-5.3	-2.8	-7.8	-6.3	-2.0
9	-20.1	-13.4	-8.7	-10.3	-4.3	-7.4	-0.04
10	-9.2	-8.4	-20.8	-13.0	-17.0	-13.0	-5.2
MPE (%)	**8.5**	**4.1**	**-0.5**	**1.5**	**0.2**	**1.3**	**4.1**
95% CI	-4.7 – 13.2	-4.1 – 8.2	-9.1 – 8.6	-5.6 – 7.2	-6.2 – 6.4	-5.0 – 6.3	-0.2 – 4.2
MAPE (%)	**18.6**	**10.9**	**10.4**	**8.7**	**7.7**	**7.6**	**6.1**

MPE = mean prediction error, **MAPE** = mean absolute prediction error

3.4.4 External validation with Bayesian procedure

The pharmacokinetic values from the final population model were used as priors for a MAP Bayesian estimator of individual pharmacokinetic parameters, based on limited-sampling strategy. The individual predicted AUC_{0-12} is presented in table 13, with mean bias (MPE) and prediction (MAPE) for the 10 new patients.

Table 13: Bayesian AUC_{0-12} (µg/L*h) estimates using different sampling times compared to observed AUC_{0-12} (µg/L*h).

	OBS	**No info**	**C_0**	**C_2**	**C_0+C_2**	**C_1+C_2**	**C_0+C_1+ C2**	**C_0+C_1+ C3**
Patient A	7408	8456	7170	8227	7362	8107	7166	7193
Patient B	8749	9892	9746	7624	8907	8012	8778	9450
Patient C	9029	11197	10260	9335	9719	9568	9408	9237
Patient D	10358	8111	8282	10085	8960	10944	9678	10920
Patient E	7255	7919	6192	9329	7351	7351	7535	7002
Patient F	6865	10903	8129	7989	7246	8158	6936	7084
Patient G	6147	8510	6423	7723	6484	7635	6126	6827
Patient H	5437	6301	4760	4969	4331	4693	4392	5364
Patient I	9646	13207	9615	9847	8725	10536	8070	8790
Patient J	6232	6349	6242	6861	6456	6538	6277	6315
MPE (%)		**19.1**	**-0.3**	**7.3**	**-1.9**	**5.8**	**-3.7**	**1.5**
95% CI		5.3 – 12.8	-8.1 – 7.6	-1.2 – 15.8	-7.7 –4.0	-1.2 –12.7	-8.6 - 1.5	-2.2 - 5.2
MAPE (%)		**23.4**	**9.9**	**12.1**	**7.0**	**10.2**	**5.6**	**4.4**

OBS: Observed AUC_{0-12}, **MPE** = mean prediction error, **MAPE** = mean absolute prediction error

The mean prediction error (MPE) and mean absolute prediction error (MAPE) were in accordance with the predictive performance from the datasplitting analysis. The MAPE was 23.4% without any concentrations given in the dataset. As in the data splitting analysis, this was due to over-prediction of the observed concentrations (+19% bias). When only C_0 concentration was provided the MAPE error was reduced to about 10%, which was slightly smaller than using C_2 (12%). Providing the model with both C_0 and C_2 reduced the MAPE to 7%, with low bias (-1.9%). The use of C_1 and C_2 did not give any notably reduction in MAPE compared to using only one time measurement. However, the plot of observed concentrations versus individual predicted concentrations (figure 11) showed good correlation (r^2=0.956), which was better than using C_0+C_2 and even better than C_0+C_1+C_3. The prediction in individual AUC_{0-12} was improved when using three time measurements, and the observed concentrations correlated well with the individual predicted concentrations. The coefficient of

determination (r^2) was about the same in the plots of observed versus predicted concentrations (figure 11) using none concentrations or C_0/C_2.

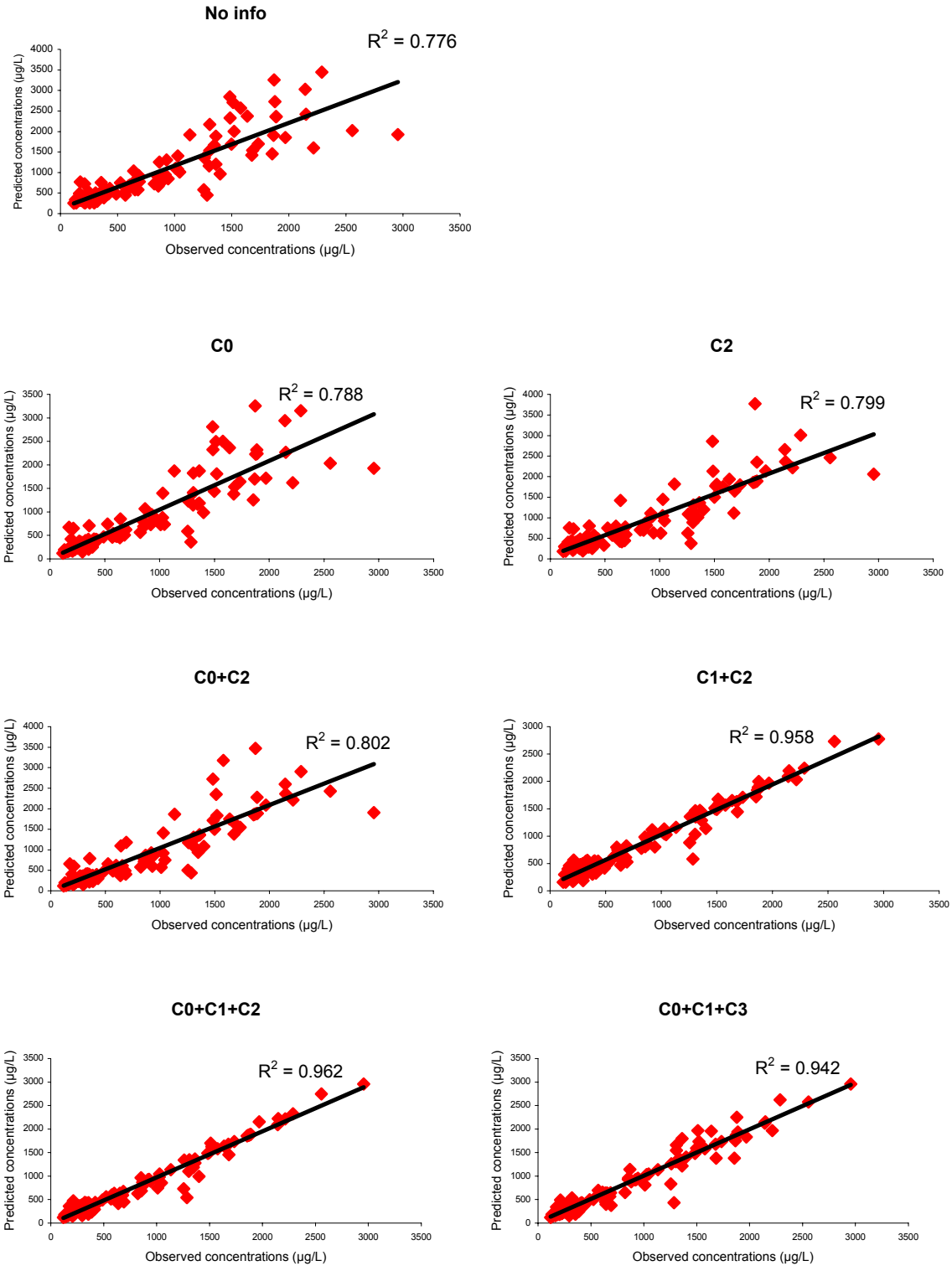

Figure 11: Scatter plots of the concentrations predicted using different Bayesian estimators versus the observed concentration in the external patient group.

Line of identity (——).

3.5 NON-POPULATION ANALYSES

The mean pharmacokinetic parameter estimates from the non-compartmental analysis and the WinNonlin analysis are presented in table 14.

Table 14: Pharmacokinetic estimates from non-compartment and WinNonlin analysis

Non-compartment				WinNonlin			
CL/F (L/hrs)	**%CV**	**V_d/F (L)**	**%CV**	**CL/F (L/hrs)**	**%CV**	**V_d/F (L)**	**%CV**
23.4	37.2	254	65.3	11.6	59.8	243	103

CL/F = apparent clearance, **V_d/F** = apparent distribution volume, **F** = bioavailability, **CV** = coefficient of variation

WinNonlin was not able to analyze the concentration-time profiles for three of the patients when using the 2-compartment model with a lag-time from the library in WinNonlin (patient 11, 14 and 34). The absorption phase was also poorly described.

3.5.1 Comparison between non-compartment analysis, WinNonlin and NONMEM

The individual estimates of CL/F and V_d/F for the three different methods was compared using SPSS. The statistical analysis in SPSS showed a significant difference between the three methods in estimating clearance, but not in estimating distribution volume (table 15).

The difference between WinNonlin and NONMEM in calculating means of V_d/F was 35%, and the difference between non-compartment analysis and NONMEM was 32%. The difference between non-compartment and WinNonlin was only 4.2%. In the calculation of mean CL/F, there were a difference of 49% between WinNonlin and NONMEM. The difference between non-compartment and NONMEM was 7.4%. In contrast to estimation of V_d/F, the difference was high between the non-compartment and WinNonlin analysis for the estimation of mean CL/F (50%).

Table 15: P-values from the statistic tests

	CL/F (L/hrs)	**V_d/F (L)**
P-value	<0.05	0.168

4 DISCUSSION

4.1 POPULATION MODELS

As described in section 1.4.8, one-, two-, and three compartment models have successfully been used to fit CsA datasets. Which model that best fits the data, may largely depend on the number of patients (and blood samples) in the population. As a general rule, at least one blood sample per patient per parameter (thetas, omegas and sigmas) is needed to be able to describe all the parameters in a model.

In all the 1-compartment models tested, NONMEM was not able to describe the highest observed concentrations, and in the elimination phase the concentrations were over-predicated. The diagnostic plot of WRES versus time showed a u-shaped curve, indicating bias in the model, which is indicative of model misspecification. The reason for the bias is the poor description of the distribution phase in a 1-compartment model, since CsA is highly lipophilic and therefore accumulates in fat-rich tissues [33]. Addition of a peripheral compartment improved the accuracy, reduced OFV and residual error. There is however studies that have chosen a 1-compartment model to fit CsA data [60, 61], but none of these studies reports wheter other compartment models have been evaluated.

The 2-compartment models tested (with exception of the model with zero order absorption) showed high correlations between observed and predicted concentrations, as shown in figure 5. The 3-compartment model with lag-time was highly sensitive for the initial parameter estimates. This is most likely due to a low number of patients (and blood samples) in proportion to number of parameters in a 3-compartment model. Moreover, the value of V3 was unlikely high. However, the predicted concentrations correlated well with the observed concentrations, and OFV and residual error were similar to the best 2-compartment models.

Based on the aspects above, a 2-compartment model for CsA seems to be a reasonable approximation for describing the pharmacokinetics of CsA. A more data-rich population is probably necessary if a 3-compartment model would be used to fit the data. Even though the OFV was significant better with the 2-compartment model, it is unknown whether the 3-compartment model reached its minimum OFV or not, since this model was highly unstable. Therefore, the effect of an additional peripheral compartment can not be completely

evaluated. A study by Fanta et al. found that a 3-compartment model best described the pharmacokinetics of CsA in a dataset consisting of 162 children (approximately 10 samples per patient) [64]. However, a study by Saint-Marcoux et al. including almost the same number of patients (147) and same number of samples per patient found that a 2-compartment model best described the CsA pharmacokinetics [63]. The in-consistent reporting on the best compartment model for CsA indicates that both a 2-compartment and a 3-compartment model may describe the pharmacokinetics of CsA.

The absorption profile of CsA is characterized by a lag-phase followed by rapid absorption, which also was present in the concentration-time profiles of the patients studied in this thesis (figure 2). The absorption phase was poorly described in the models that did not account for a delay in the absorption (figure 5). The concentrations were over-predicted in the beginning of the absorption phase, followed by an under-prediction of the concentrations around C_{max}. NONMEM assumes rapid absorption when no lag-time is present in the model, and as a consequence of this over-prediction in the beginning of the absorption the concentrations around C_{max} is under-predicted. The fit around C_{max} was better when the absorption phase was adequately described.

A zero order rate constant did not describe the absorption of CsA, which may indicate that the absorption of CsA is dependent of the amount of drug remaining to be absorbed. However, Bourgoin et al. reported a model with zero order absorption (and lag-time) to best describe the CsA dataset [62], and both zero- and first- order absorption kinetics were evaluated in this study. There is, however, a main emphasis for using first order absorption kinetics to describe the pharmacokinetics of CsA [60, 61, 64].

Including lag compartments (Erlang distribution) in the absorption phase gave a better fit of the CsA data than a classical zero- or first-order rate constant connected with a lag-time parameter. Even though the change in OFV was not significantly lower compared to the 2-compartment model with first order absorption and lag-time, the 2-compartment model with Erlang distribution was more robust. The estimates of the pharmacokinetic parameters in the 2-compartment model with Erlang distribution did not change significantly when changing the initial estimates, while the estimates in the other models were highly unstable. This is probably a result of the greater flexibility of the model with Erlang distribution when

modeling flat/delayed absorptions profiles. Previous studies have also proposed models including serial lag compartments (Erlang distributions) to predict highly variable absorption processes [63, 66, 81], demonstrating an advantage in such a model when modeling flat/delayed absorption. Furthermore, the 2-compartment model with Erlang distribution required estimation of 5 pharmacokinetic parameters (CL, Q, V_c, V_p and k_{tr}) compared with 6 parameters for the 2-compartment model with lag time (CL, Q, V_c, V_p, k_a and lag time). This difference is important considering the fact that the more parameters in the model the more samplings times are required.

The average values of the pharmacokinetic parameters obtained in this thesis were similar to those published in previously studies in renal transplant patients using a 2-compartment model with Erlang distribution to describe the absorption phase [63, 66, 81]. In addition, the average values of the parameters were close to those previously published using a 2-compartment model without Erlang distribution [62, 65].

The estimated interindividual variability is a measure of the unexplained random differences between individuals, and the mean values of the interindividual variability in this thesis were consistent with previous results. Interindividual variability in CL/F, Q/F, K_{tr} and V_C/F was moderate, whereas interindividual variability in V_P/F was high (95.6%). However, the high interindividual variability in V_P/F is comparable to previously reports. Hesselink et al. reported an interindividual variability in V_C/F of 128% [65], Saint-Marcoux et al. reported an interindividual variability in V_P/F of 80% [63] and Fanta et al. reported an interindividual variability in V_C/F of 124.4% [64].

Residual variability represents the uncertainty in the relationship between the blood concentrations predicted by the model and the observed concentration. Modeling residual variability as a combination of additional and proportional error model gave the values 37.6 µg/L and 13.5% respectively. These findings are in accordance with previously work [60, 63, 66]. Correct measurement of the magnitude and structure of the residual error may be important if the model is to be used as prior information for subject-specific Bayesian estimation. However, there is difficult to evaluate which error model that describes the residual variability of the data best, since the true value of the residual error is not known.

Predicted (PRED) and individual predicted (IPRED) concentrations versus observed (OBS) concentrations were randomly distributed around the line of identity, and did not show any clear bias (figure 7). This indicates that the model works, with no suggestion of model misspecification. The correlation (r^2) was better between IPRED and OBS than between PRED and OBS. This is because IPRED are based on individual models for each patient, in stead of mean parameter values calculated for the whole population.

The scatterplot of weighted residual error (WRES) versus time were uniform spread without any trend (figure 7). The scatterplot of WRES versus ID showed an indication of an outlier. This patient (ID number 10) had an observed concentration of 3766 μg/L, which is higher than the standard curve in the method used for analysing CsA concentrations [82]. Therefore, the large WRES in this patient may be due to higher variation in the whole blood analysis of this concentration.

4.2 COVARIATE ANALYSIS

The different population analyses of CsA using NONMEM report different covariates for significant influence on the pharmacokinetic parameters. Low number of patients included in a study may hinder proper statistics, as may be the case in this thesis. However, a study by Kyhl et al. including 728 stable kidney transplant patients [83] showed no effect of age, gender, dose, height, days since transplantation or weight on the pharmacokinetics of CsA. These findings suggest that other factors, like genetic polymorphism, may contribute to variability in CsA pharmacokinetics. The association between genetic factors in the metabolic-and transport enzymes and absorption/ clearance of CsA has been investigated [65, 84-87]. However, no clear differences were demonstrated in these studies, even though a tendency for a correlation between the expression of *CYP3A5*1* and higher metabolism was observed. Haufroid et al. [86] and Hu et al. [87] reported higher dose-adjusted trough concentrations (C_0) in patients expressing the *CYP3A5*1*, which is expressed in the liver of approximately 20% of the population [88]. Further studies are needed to explore this relationship. In this thesis, none of the patients expressed the *CYP3A5*1* allele, so the relationship could therefore not be examined.

Age as a covariate on CL/F was the only covariate that gave significant lower OFV. CsA is primarily eliminated via cytochrome P450(CYP)3A biotransformation in the liver and small intestine [26, 30]. No age-related decrease in the CYP3A activity has been reported either *in vitro* or *in vivo* [89]. By contrast, a significant fall in liver mass and liver blood flow with age has been documented [90]. For a drug with high clearance intrinsic, like CsA, the effect of age on elimination is therefore expected. The interindividual variability in clearance was not reduced notably when including age as a covariate for clearance, which may indicate that the effect of age was slightly. However, the interindividual variability in the peripheral distribution volume (V_P/F) was reduced from 110% to 96%.

Body weight during CsA treatment is an important aspect, since many patients gain weight after transplantation. The increase in body weight is due to re-establishing an anabolic state and administration of high-dose steroid. Previous works have found an effect of body weight on distribution volume (V_d/F) [60, 61, 91]. Introducing body weight as a covariate on V_d/F gave a non-significant reduction in OFV. Moreover, the interindividual variability in distribution volume (V_d/F) was not reduced when adding weight as a covariate on V_d/F; in fact it was increased by 2.5%. However, the small number of patients may have hindered proper statistical evaluation. In addition, the range in body weight was low [68-97], with a SD of 9 kg, which may have further contributed to a non-significant result.

The lack of significant influence from estimated creatinine clearance on CsA is logical considering that CsA is primarily eliminated by metabolism [26, 30], which means that decreased renal function does not affect its pharmacokinetics considerably.

It has been demonstrated in studies that diabetics have a slow and erratic absorption of CsA, with more intrapatient variability in C_2 [75]. Four of the patients in this thesis were diabetic. By visual examinations of the concentration-time curves, only one of these patients showed an indication of a more slow absorption. In this thesis the patients with diabetes did not show a relevant slower absorptions profile. The estimated transfer constant (k_{tr}) for the diabetic patients was 7.84, compared to 7.87 in non diabetic patients. However, the number of diabetic patients was too small to give any significant differences, although there was a tendency of no difference between diabetic and non-diabetic patients with regards to absorption.

When modeling slow absorptions profile using the mixture function, NONMEM placed only one patient in the subpopulation with slower absorption (lower k_a). Interestingly, this patient was non-diabetic. The very slow absorption of this patient is suspected to be due to eating prior to CsA morning dose. However, this aspect is important for further investigation in order to improve the model; can another covariate be added to the model to better describe the slow absorption?

Previous work has reported that the value of CL/F decline after transplantation, especially within the 3 first weeks [60, 91]. The mean post-transplantation period for patients studied here were 5.5 weeks [2.1-10.4], with only 3 patients within the 3 first weeks after transplantation. From the graphical analysis, CL/F showed an indication of a higher value within the 3 first weeks (appendix). In the studies reporting a decline in CL/F after transplantation more patients and a wider range in post-transplantation period were present. If more patients within the 3 first weeks after transplantation were included in the dataset, a time-related clearance could perhaps improve the model.

Konishi et al. have demonstrated that treatment with steroid (methylprednisolone sodium succinat) significantly increased the total body clearance of intravenously administration of CsA by induction of hepatic CYP3A [92]. In addition, systemic bioavailability of CsA after oral administration were shown to be markedly reduced by steroid dosing, and the mechanism of interaction was confirmed to involve enhancement of P-gp and decrease in bile secretion [93]. The effects of steroid dose are more prominent the first time after transplantation, since dosing of steroid are higher initially. No clear relationship was found in this thesis. However, an indication of a higher clearance associated with a 20 mg dose of steroid at the pharmacokinetic day compared to 10 mg dose was seen (appendix).

Gender had no effect on any of the pharmacokinetic parameters. No clear relationships were seen in the graphical covariate analysis (appendix). It has been shown that females have a higher CYP3A activity than males [94], which could result in higher clearance of CsA in females. In fact, the tendency was opposite here; a slightly higher clearance for men was seen in the graphical analysis (appendix). However, this incompatible relationship is probably caused by a low number of females (6/17), and was therefore not tested any further. The

effect of height was also insignificant, which was not surprising considering the fact that weight did not influence the distribution volume in this thesis.

4.3 VALIDATION

The posterior predictive check method gives an initial quantitative validation of the model. The result did not give any suspicion of model misspecification, since the 95% CI of C_{max}, C_{trough} and AUC_{0-12} from the 100 simulations contained the mean of the "true" values. In addition, the paired statistic test showed no significant differences between observed and simulated values of C_{max}, C_{trough} and AUC_{0-12}.

A data splitting analysis was further applied. This approach is recommended by the US Food and Drug Administration (FDA) [7]. The pharmacokinetic parameter estimates in the subset groups were not significantly different from those obtained from the whole data set, which indicates that no subsets of the population had high influence on the estimation of the pharmacokinetic parameters. Moreover, the OFVs obtained by another NONMEM run for the full data set fixing the parameter estimates for the subsets were not significant different from the OFV in the final model ($\Delta OFV = 0.9$). The data splitting analysis confirmed the robustness of the final model.

The predictive performance of the 10% of patients excluded in each of the 10 subset groups showed a good prediction of individual AUC_{0-12}. Predicting AUC_{0-12} using the population model with individual dose and age provided, resulted in an absolute error in prediction of 18.5%, which is relative low considering the limited information given (dose and age). In addition, this result is in agreement with a data splitting analysis for CsA performed by Saint-Marcoux et al., which reported a mean absolute prediction error (MAPE) of 18% [63]. Irtan et al. studied pharmacokinetics of CsA in pediatric renal transplant patients, and found a MAPE of 29.4% in a data splitting procedure [81]. When including one time measurement (C_0 or C_2), the prediction error was reduced to an average of 10.5%, with no clear difference between C_0 and C_2. As expected, the predictions were better when including two or three measurements within the absorption phase. These results demonstrate the good performance of the population model developed, which was further supported by testing the model in an external group.

The predictive performance in an external group consisting of 10 new kidney transplant patients was also tested. Providing the model with information about concentrations at 0, 1 hour and 3 hour provided the best prediction of individual AUC_{0-12} (4.79%), which is in agreement with previously Bayesian estimation studies. Saint-Marcoux et al. reported a MAPE of 10.5% [63], Rousseau et al. reported a MAPE of 5.3% [66] and Leger et al. reported a MAPE of only 2% [95] when using a Bayesian estimator at times 0, 1 hour and 3 hour. Bourgoin et al [62] selected times 0, 1 hour and 2 hour for Bayesian estimation, and found an accuracy of 13.1%.

The purpose of Bayesian estimation is to apply it to AUC-based TDM of CsA, and therefore practicality is important. Using only one concentration-measurement provided, in clinical terms, good prediction of observed AUC. A MAPE of approximately 10% was observed using C_0, while the MAPE was approximately 12% when using C_2. A MAPE of 10-12% should not have important clinical consequences, with respect to proposed therapeutic range for CsA. Mahalti et al. suggest a target AUC_{0-12} in kidney transplant patients in the range of 9500-11500µg*h/L during the first period after transplantation [96]. However, target AUC may differ according to different authors.

External validation is the most stringent test of a model. Bayesian method using limited blood samples allowed a precise estimation of AUC_{0-12} in a population of 10 kidney transplant recipients. In addition, the results in the external group were in agreement with the internal validation method. However, for clinical purposes, the model should be able to predict individual AUC_{0-12} the day after the time measurement(s).

4.4 NON-POPULATION ANALYSES

A WinNonlin analysis and non-compartment calculation in Excel were performed to elucidate whether there were significant differences between the parameter estimates obtained in these methods and the NONMEM analysis. The result showed a significant difference in estimating CL/F, but not in V_d/F. However, the large variation seen in V_d/F (22–1212L) makes it difficult to truly evaluate statistic significant differences. An interesting finding was that non-compartment calculations were closer to the NONMEM analysis in estimating V_d/F and CL/F compared to the WinNonlin analysis.

Regardless of significant differences in parameter estimates or not, population analysis (NONMEM) has advantages over the two other methods in estimating variability, considering variance of point estimates and allowing formal testing of covariates. The variance of point estimates are important, especially if the data set are small and simultaneously contains outliers. In addition, Bayesian approach diminishes importance when not doing a population analysis as performed with NONMEM.

The WinNonlin analysis would have been more valuable if it were performed before the NONMEM analysis. WinNonlin results can serve as indication of initial parameter estimates for the population modeling. In addition, individual modeling in WinNonlin can give a good suggestion for the most likely compartment model for the dataset.

5 CONCLUSION AND FUTURE CONSIDERATIONS

The main aim for this thesis was to develop a pharmacokinetic population model for CsA, which in the future can be used as a Bayesian prior when designing dosing regimens for new kidney transplant recipients.

In order to find the best pharmacokinetic population model, different compartment models with different absorption profiles were examined. From the different models tested, it can be concluded that a 2-compartment model with Erlang distribution to describe the absorption phase provided the best fit of the CsA data set.

In the screen for patient covariates that could describe some of the interindividual variability in the pharmacokinetic parameters, it can be concluded that age was a significant covariate for clearance. However, there is reason to believe that the data set used for this purpose was too sparse for other covariates to reach statistic significance. A re-run of the covariate analysis including more patients is therefore needed.

Finally, the model was also validated with both internal and external methods. The results indicated that the pharmacokinetic population model developed is robust and that the model is able to predict individual $AUC_{0\text{-}12}$ in new kidney transplant patients using limited concentration measurements, with no clear differences from the internal validation method. However, more patients included in the dataset would confirm the predictive performance of the population model. Furthermore, the model should be able to predict individual $AUC_{0\text{-}12}$ the day after the time measurement(s) for practical use in clinical settings. For this purpose, prior dose history needs be included in the dataset when developing the pharmacokinetic population model and the effect of inter-occasion variability should be evaluated.

In conclusion, a 2-compartment model with Erlang distribution as an absorption process and age as a covariate provides a good basis for the development of a model that can be used to optimize dosing regimens in new kidney transplant patients.

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7 APPENDIX

7.1 Input file for building the population model

ID	AMT	RATE	TIME	CON	WT	CRCL	MDV	SS	II	CMT	FLAG	AGE	GENDER	HEIGHT	TXT	STER
7	100000	0	0	0	70.2	43	1	2	12	1	1	60	2	1.65	9	10
7	0	0	0	380	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	0.23	451	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	0.58	775	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	1.13	1639	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	1.62	2063	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	2.07	1217	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	3.1	692	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	4.03	625	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	6.02	454	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	8.02	385	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	9.97	315	70.2	43	0	0	0	8	1	60	2	1.65	9	10
7	0	0	11.93	312	70.2	43	0	0	0	8	1	60	2	1.65	9	10
8	225000	0	0	0	90.3	95.2	1	2	12	1	1	59	1	1.85	4.1	20
8	0	0	0	383	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	0.23	274	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	0.48	764	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	0.98	1633	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	1.43	1941	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	1.98	1976	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	3.03	944	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	4.07	681	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	5.98	574	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	8.03	319	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	9.97	187	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
8	0	0	11.9	262	90.3	95.2	0	0	0	8	1	59	1	1.85	4.1	20
9	200000	0	0	0	75.7	53.5	1	2	12	1	1	33	2	1.8	7.6	20
9	0	0	0	227	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	0.12	180	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	0.45	334	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	0.97	1666	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	1.47	1452	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	1.95	1227	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	2.92	754	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	3.88	393	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	5.9	197	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	7.88	267	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
9	0	0	11.62	155	75.7	53.5	0	0	0	8	1	33	2	1.8	7.6	20
10	150000	0	0	0	67.6	52.5	1	2	12	1	1	35	1	1.85	4.6	15
10	0	0	0	260	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	0.3	273	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	0.52	690	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	0.98	2766	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	1.48	3766	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	2.02	2402	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	3	1748	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	3.95	1461	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	5.88	590	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15

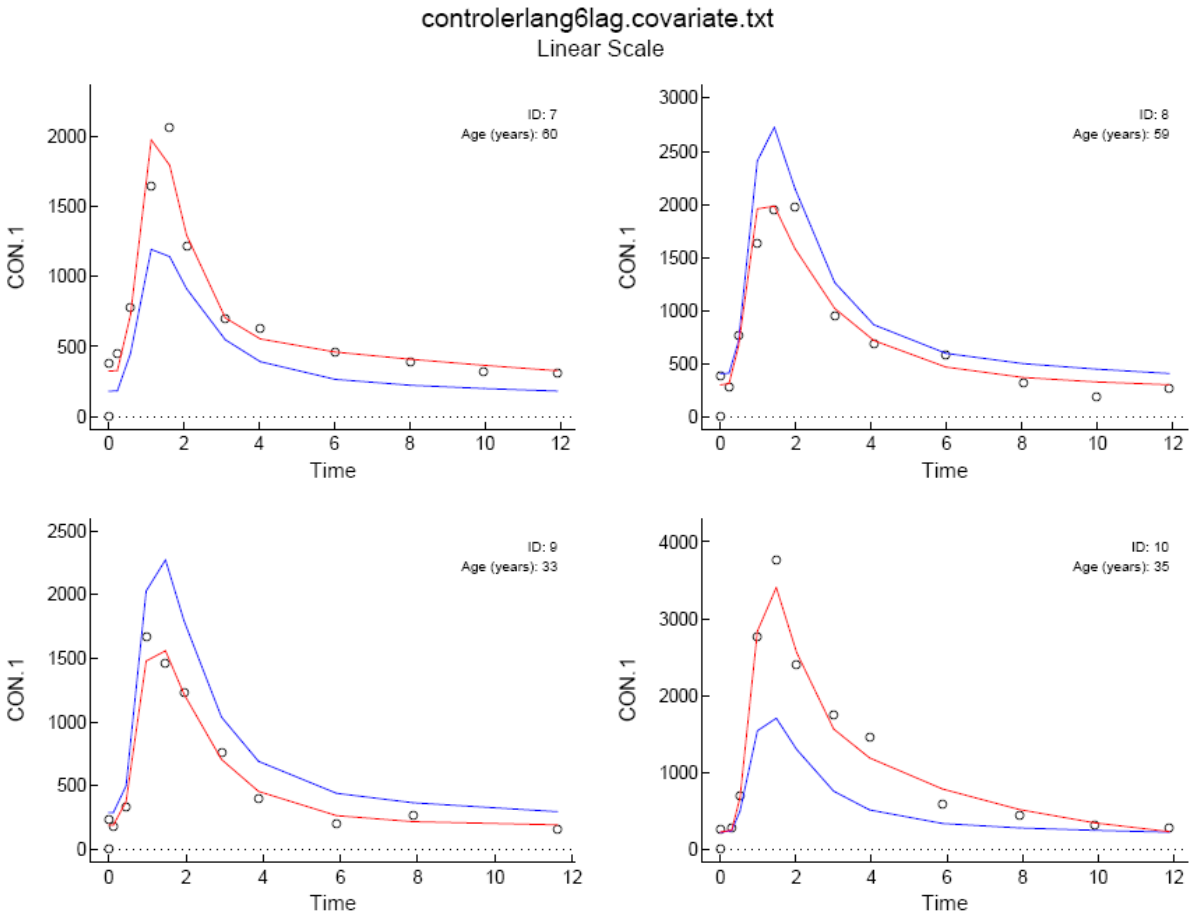
10	0	0	7.92	428	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	9.9	314	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
10	0	0	11.88	267	67.6	52.5	0	0	0	8	1	35	1	1.85	4.6	15
11	200000	0	0	0	75.3	81.2	1	2	12	1	1	52	1	1.88	4.4	20
11	0	0	0	364	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	0.3	313	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	0.57	371	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	1	2575	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	1.52	2084	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	2.03	1690	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	2.97	1522	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	3.95	1001	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	5.97	524	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	7.87	552	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	9.95	407	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
11	0	0	11.75	359	75.3	81.2	0	0	0	8	1	52	1	1.88	4.4	20
12	225000	0	0	0	96.7	72.2	1	2	12	1	1	67	1	1.81	4.3	15
12	0	0	0.25	510	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	0.48	942	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	1.18	1669	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	1.65	1830	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	2.17	1732	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	3.18	864	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	4.13	711	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	6.13	622	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	8.08	415	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	10.02	377	96.7	72.2	0	0	0	8	1	67	1	1.81	4.3	15
12	0	0	11.97	343	96.2	72.2	0	0	0	8	1	67	1	1.81	4.3	15
14	125000	0	0	0	68.7	92.7	1	2	12	1	2	60	2	1.72	6.3	15
14	0	0	0	257	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	0.2	261	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	0.48	389	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	1.07	2398	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	1.55	1709	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	2	1492	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	3	919	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	3.97	624	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	5.95	488	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	8	312	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	10.03	246	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
14	0	0	11.73	178	68.7	92.7	0	0	0	8	2	60	2	1.72	6.3	15
18	125000	0	0	0	74.1	54.8	1	2	12	1	1	74	1	1.64	3.1	20
18	0	0	0	325	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	0.25	285	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	0.53	460	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	1.13	1888	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	1.6	2299	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	2.07	1391	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	3.05	884	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	4.03	564	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	6	384	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	8.08	301	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	10.12	239	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20
18	0	0	11.8	205	74.1	54.8	0	0	0	8	1	74	1	1.64	3.1	20

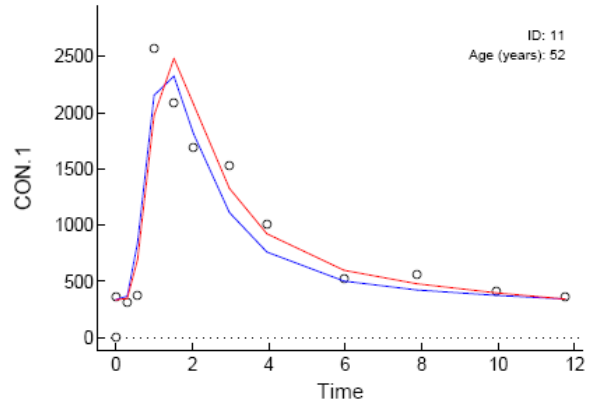
19	350000	0	0	0	79.9	63.5	1	2	12	1	2	52	1	1.76	2.1	20
19	0	0	0	340	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	0.23	366	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	0.48	343	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	1.08	1814	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	1.55	1923	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	2.05	1480	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	3.03	1192	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	4.02	953	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	5.98	782	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	8.1	462	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	10.1	435	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
19	0	0	11.78	407	79.9	63.5	0	0	0	8	2	52	1	1.76	2.1	20
30	150000	0	0	0	91.5	75.3	1	2	12	1	1	25	1	1.82	9.3	10
30	0	0	0	153	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	0.5	247	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	1.03	783	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	1.52	1041	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	2.02	1266	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	2.52	939	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	3.05	825	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	4.25	474	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	4.97	355	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	6.03	259	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	7.98	215	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	9.98	157	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
30	0	0	11.9	126	91.5	75.3	0	0	0	8	1	25	1	1.82	9.3	10
31	125000	0	0	0	78	74.1	1	2	12	1	1	61	2	1.7	10.4	20
31	0	0	0	346	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	0.6	1104	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	1.2	1743	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	1.52	1408	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	2	994	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	2.53	718	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	3.02	580	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	4.05	428	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	4.97	392	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	6.03	294	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	8.07	276	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	10.07	269	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
31	0	0	12.02	263	78	74.1	0	0	0	8	1	61	2	1.7	10.4	20
32	150000	0	0	0	90.8	86.7	1	2	12	1	2	59	1	1.79	9	20
32	0	0	0	173	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	0.48	287	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	1.08	1165	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	1.53	1782	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	2.07	1169	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	2.53	1095	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	3.03	1028	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	4	639	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	5.02	526	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	6.03	445	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	7.98	373	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
32	0	0	9.98	319	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20

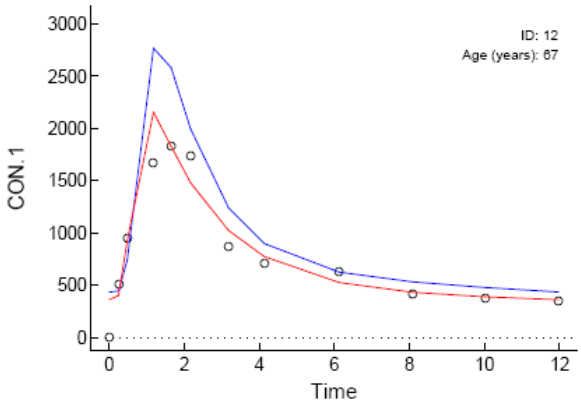
32	0	0	11.68	316	90.8	86.7	0	0	0	8	2	59	1	1.79	9	20
33	175000	0	0	0	78	69.4	1	2	12	1	2	68	2	1.56	3	20
33	0	0	0	317	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	0.5	1260	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	1	2492	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	1.53	2046	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	2	1862	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	2.55	1413	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	3.02	1217	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	3.97	787	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	5.02	739	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	6	585	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	8.05	543	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	10	487	78	69.4	0	0	0	8	2	68	2	1.56	3	20
33	0	0	11.53	404	78	69.4	0	0	0	8	2	68	2	1.56	3	20
34	125000	0	0	0	85.7	66.2	1	2	12	1	1	69	2	1.64	6.9	15
34	0	0	0	367	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	0.57	1235	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	1.05	2387	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	1.53	1801	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	2.02	1000	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	2.53	829	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	3.03	613	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	3.98	633	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	4.97	552	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	6.02	376	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	8.03	310	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	10	329	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
34	0	0	11.95	341	85.7	66.2	0	0	0	8	1	69	2	1.64	6.9	15
35	250000	0	0	0	80	100.4	1	2	12	1	1	23	1	1.8	4	15
35	0	0	0	348	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	0.5	354	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	1.03	609	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	1.55	1175	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	2.05	1530	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	2.55	1760	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	3.1	1406	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	4.03	1034	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	5	815	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	6	575	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	8	383	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	9.98	333	80	100.4	0	0	0	8	1	23	1	1.8	4	15
35	0	0	11.93	247	80	100.4	0	0	0	8	1	23	1	1.8	4	15
36	125000	0	0	0	86.4	76.8	1	2	12	1	1	52	1	1.89	3	20
36	0	0	0	117	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	0.48	460	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	0.98	1313	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	1.5	1583	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	1.97	797	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	2.52	505	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	3.02	450	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	4.05	335	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	5	231	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	5.98	170	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20

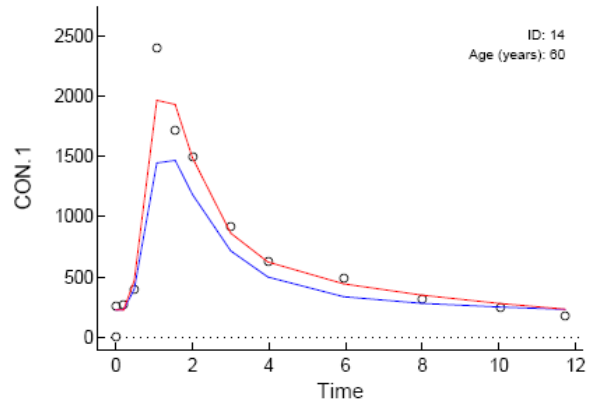
36	0	0	7.98	151	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	9.98	103	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
36	0	0	11.77	285	86.4	76.8	0	0	0	8	1	52	1	1.89	3	20
37	125000	0	0	0	85.6	74.9	1	2	12	1	1	59	1	1.89	3.3	20
37	0	0	0	190	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	0.5	848	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	1.02	2027	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	1.5	1690	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	1.98	1474	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	2.55	813	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	3.03	784	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	4.08	548	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	5	508	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	6.02	399	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	8.02	338	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	10	289	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20
37	0	0	11.8	216	85.6	74.9	0	0	0	8	1	59	1	1.89	3.3	20

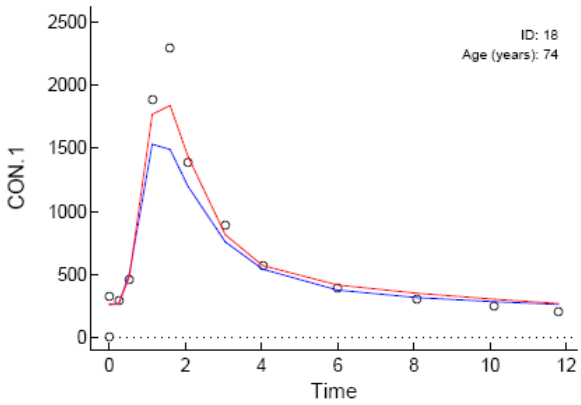
7.2 Individual fits in the final pharmacokinetic model

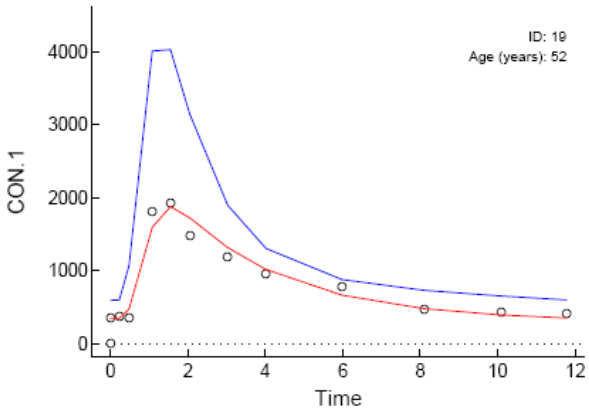


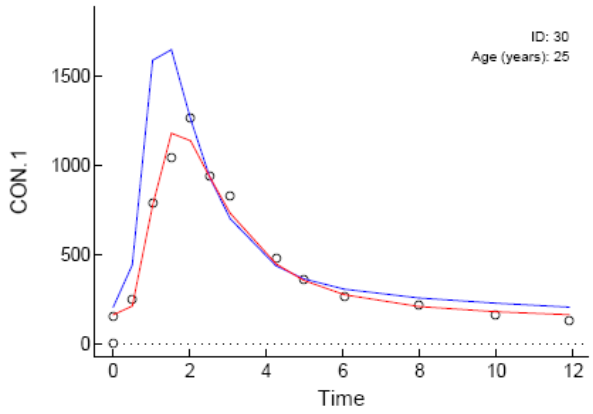

ID: 11
Age (years): 52
CON.1
Time

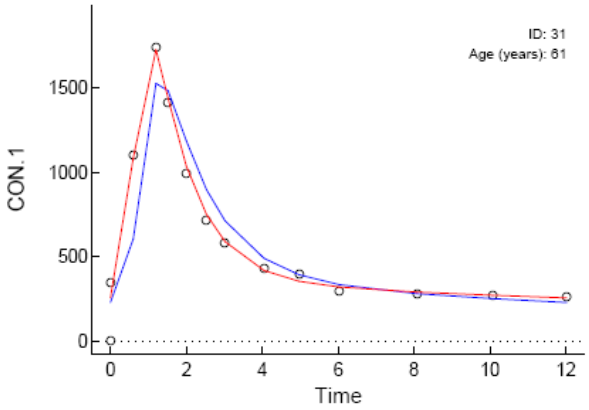

ID: 12
Age (years): 67
CON.1
Time

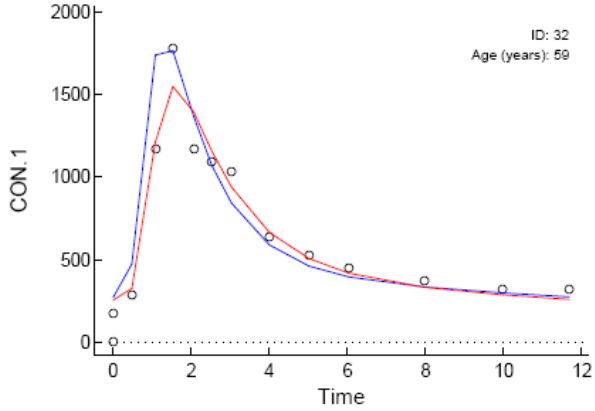

ID: 14
Age (years): 60
CON.1
Time

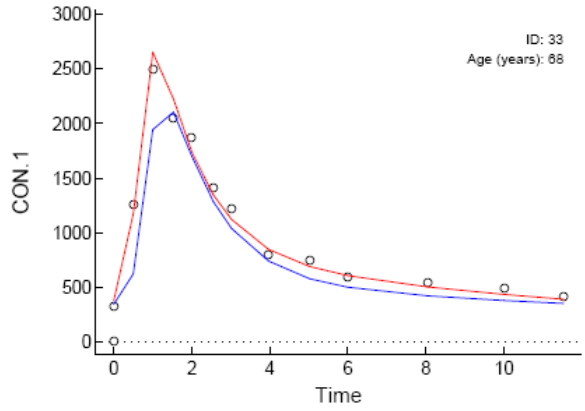

ID: 18
Age (years): 74
CON.1
Time

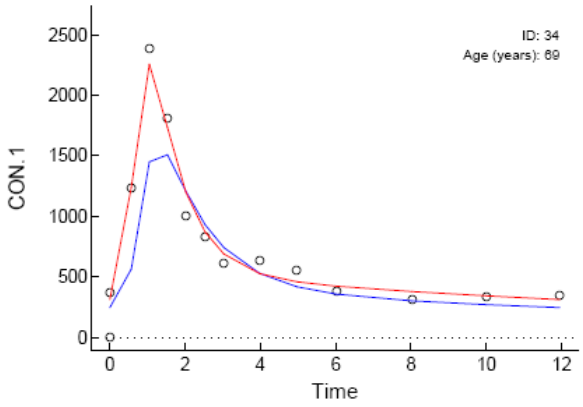

ID: 19
Age (years): 52
CON.1
Time

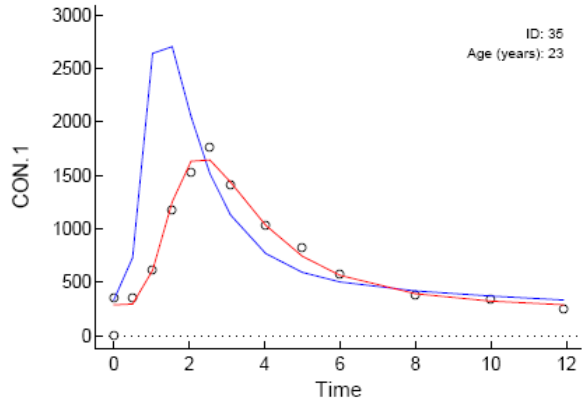

ID: 30
Age (years): 25
CON.1
Time

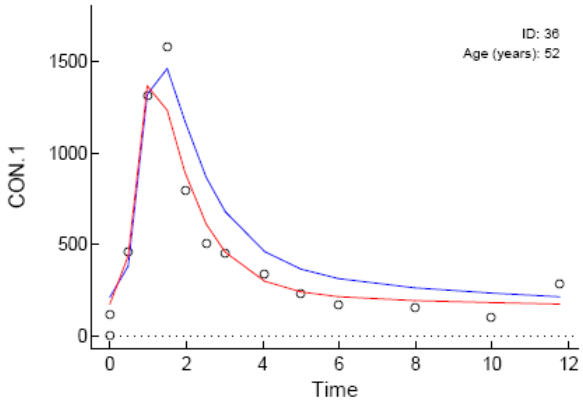

ID: 31
Age (years): 61
CON.1
Time

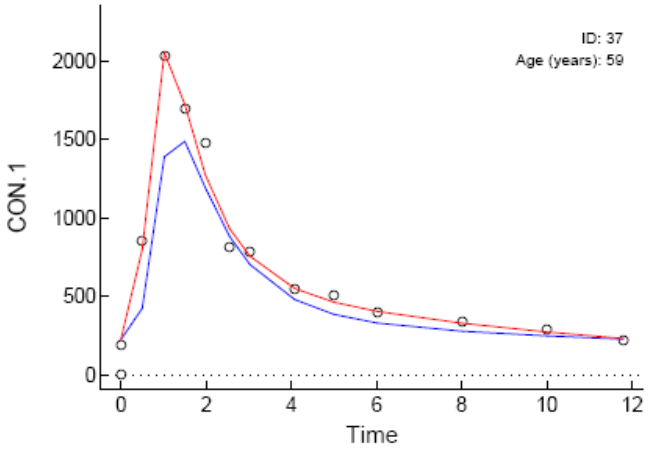

ID: 32
Age (years): 59
CON.1
Time


ID: 33
Age (years): 68
CON.1
Time

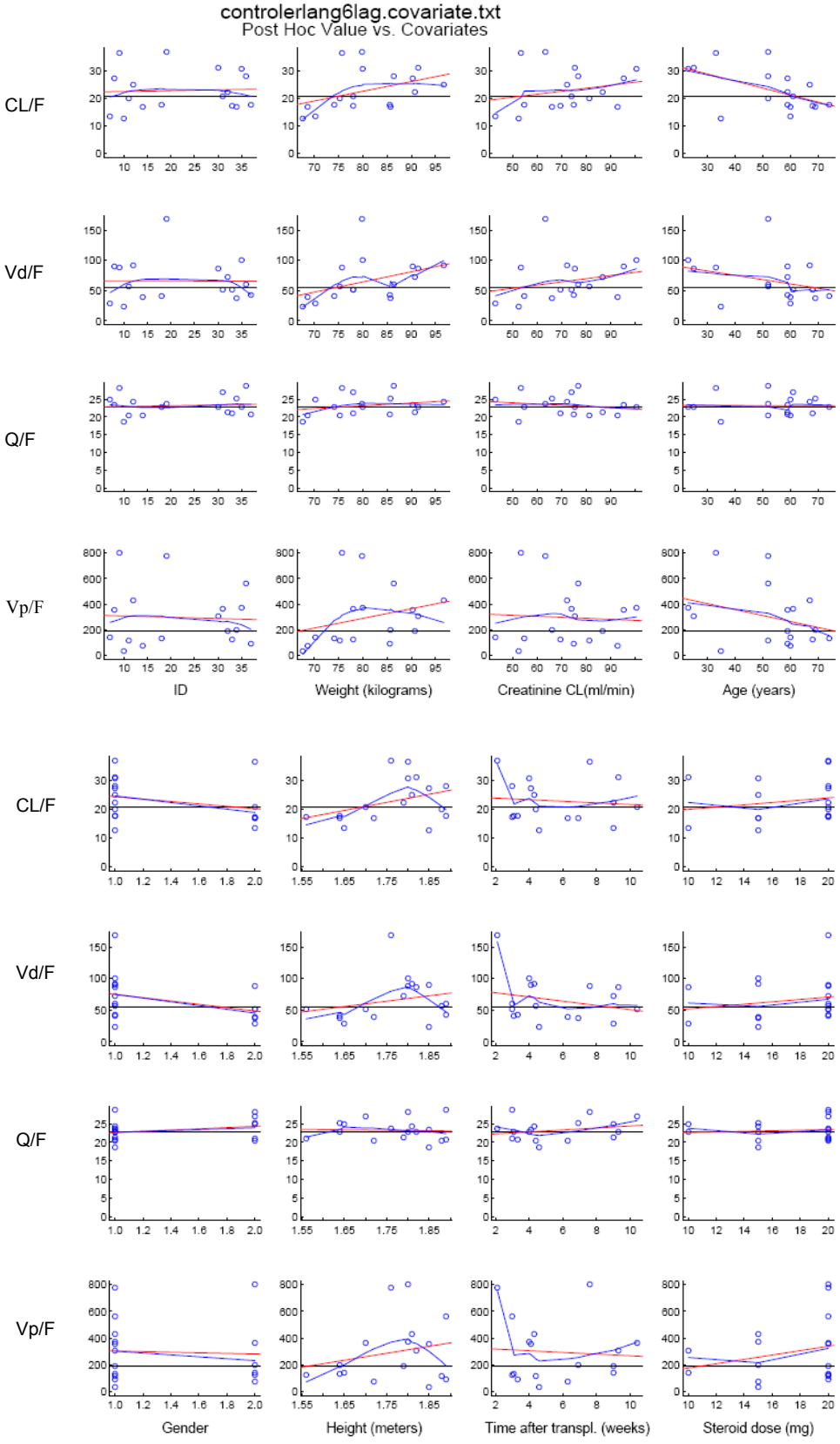

ID: 34
Age (years): 69
CON.1
Time


ID: 35
Age (years): 23
CON.1
Time


ID: 36
Age (years): 52
CON.1
Time


ID: 37
Age (years): 59
CON.1
Time

7.3 Covariate analysis



7.4 Control file for the 1-compartment model with lag-time

```
$PROBLEM 1-compartment firs order absorption with lagtime

$DATA CsA.pasienter.txt

$INPUT ID AMT RATE TIME C=DV MDV SS II

$SUBROUTINE ADVAN2 TRANS2

$PK
CL = THETA(1)*EXP(ETA(1))
V = THETA(2)*EXP(ETA(2))
KA = THETA(3)*EXP(ETA(3))
ALAG1 = THETA(4)*EXP(ETA(4))

S2 = V

$ERROR
IPRED=F
Y=F+F*ERR(1)+ERR(2)

$THETA
(1, 20)
(10,114)
(0.1, 1)
(0.1, 0.45)

$OMEGA BLOCK (3)
0.097
0.1   0.12
0.05  0.006  0.2

$OMEGA
0.02

$SIGMA 0.01 ;ERRCV
$SIGMA 10 ;ERRSD

$ESTIMATION METHOD=1 INTER MAXEVAL=9999 PRINT=1 POSTHOC

$COVARIANCE

$TABLE ID TIME DV IPRED
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID V CL ETA1 ETA2
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.5 Control file for the 2-compartment model with first order absorption and a lag-time

```
$PROB 2-compartment first order absorption with a lag-time

$DATA InputfilMEDkovariater.txt

$INPUT ID AMT RATE TIME CON=DV WT CRCL MDV SS II FLAG AGE SEX

$SUBROUTINE ADVAN4

$PK ; Define basic PK relationships
CL=THETA(1)*EXP(ETA(1))
V1=THETA(2)*EXP(ETA(2))
Q=THETA(3)*EXP(ETA(3))
V2=THETA(4)*EXP(ETA(4))
KA=THETA(5)*EXP(ETA(5))
ALAG1=THETA(6)*EXP(ETA(6))

S2=V1

K=CL/V1
K23=Q/V1
K32=Q/V2

$ERROR
IPRED=F
Y=F+F*ERR(1)+ERR(2

$THETA
(10,21)
(10,43)
(10,23.5)
(10,214,1000)
(0.1,1.52)
(0.1,0.45

$OMEGA BLOCK(6)
0.074
0.002 0.068
0.002 0.002 0.0242
0.0001 0.0001 0.0001 0.001
0.002 0.002 0.002 0.002 0.0339
0.002 0.002 0.002 0.002 0.002  0.011

$SIGMA 0.1 ;ERRCV
$SIGMA 10 ;ERRSD

$ESTIMATION NOABORT METHOD=1 INTER MAXEVAL=9999 PRINT=5 POSTHOC ;
FOCE method

$COVARIANCE

$TABLE ID TIME DV IPRED
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID V1 V2 CL Q WT CRCL AGE SEX ETA1 ETA2 ETA3 ETA4
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.6 Control file for the 2-compartment model with zero order absorption and a lag-time

```
$PROB 2-compartment zero order absorption and a lagtime

$DATA Inputfil.txt

$INPUT ID AMT RATE TIME CON=DV MDV SS II

$SUBROUTINE ADVAN3

$PK
  CL=THETA(1)*EXP(ETA(1))
  V1=THETA(2)*EXP(ETA(2))
  Q=THETA(3)*EXP(ETA(3))
  V2=THETA(4)*EXP(ETA(4))
  Ka=THETA(5)*EXP(ETA(5))
  ALAG1=THETA(6)*EXP(ETA(6))

  S2=V1

  K=CL/V1
  K12=Q/V1
  K21=Q/V2

$ERROR
  IPRED=F
  Y=F+F*ERR(1)+ERR(2)

$THETA (1,10)
$THETA (1,1)
$THETA (1,10)
$THETA (10,50)
$THETA (0.1,0.32)
$THETA (0.1,0.45)

$OMEGA
0.01
0.01
0.01
0.01
0.01
0.01

$SIGMA 0.025
$SIGMA 1

$ESTIMATION SIG=4 MAX=9999 PRINT=1 METHOD=1 INTER POSTHOC

$COVARIANCE

$TABLE ID TIME DV IPRED
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID V1 V2 CL Q ETA1 ETA2 ETA3 ETA4
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.7 Control file for the 3-compartment model with lag-time

```
$PROB Three Compartment first order absorption with a lagtime

$DATA InputfilMEDkovariater.txt

$INPUT ID AMT RATE TIME CON=DV WT CRCL MDV SS II FLAG AGE SEX

$SUBROUTINE ADVAN12

$PK ; Define basic PK relationships
  NCMT = 3
  CL=THETA(1)*EXP(ETA(1))
  V1=THETA(2)*EXP(ETA(2))
  CLRA=THETA(3)*EXP(ETA(3))
  V2=THETA(4)*EXP(ETA(4))
  CLSL=THETA(5)*EXP(ETA(5))
  V3=THETA(6)*EXP(ETA(6))
  KA=THETA(7)*EXP(ETA(7))
  ALAG1=THETA(8)*EXP(ETA(8))

  S2=V1

  K=CL/V1
  K23=CLRA/V1
  K24=CLSL/V1
  K32=CLRA/V2
  K42=CLSL/V3

$ERROR
  IPRED=F
  Y=F+F*ERR(1)+ERR(2)

$THETA
(10,20.5)
(10,43)
(10,10.1)
(10,20)
(1,15.3)
(10,605,10000)
(1,1.45)
(0.1,0.45)

$OMEGA BLOCK(6)
0.074
0.002 0.068
0.002 0.002 0.0242
0.0001 0.01  0.01  0.16
0.002 0.002 0.002 0.002 0.0339
0.002 0.002 0.002 0.002 0.002  0.0339

$OMEGA
0.1 ;BSVKA
0.01 ;BSVALAG1

$SIGMA 0.1 ;ERRCV
$SIGMA 10 ;ERRSD
```

```
$ESTIMATION METHOD=1 INTER MAXEVAL=9999 PRINT=1
POSTHOC

$COVARIANCE

$TABLE ID TIME DV IPRED NCMT
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID V1 V2 V3 CL CLRA CLSL WT CRCL AGE SEX ETA1 ETA2
ETA3 ETA4
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```